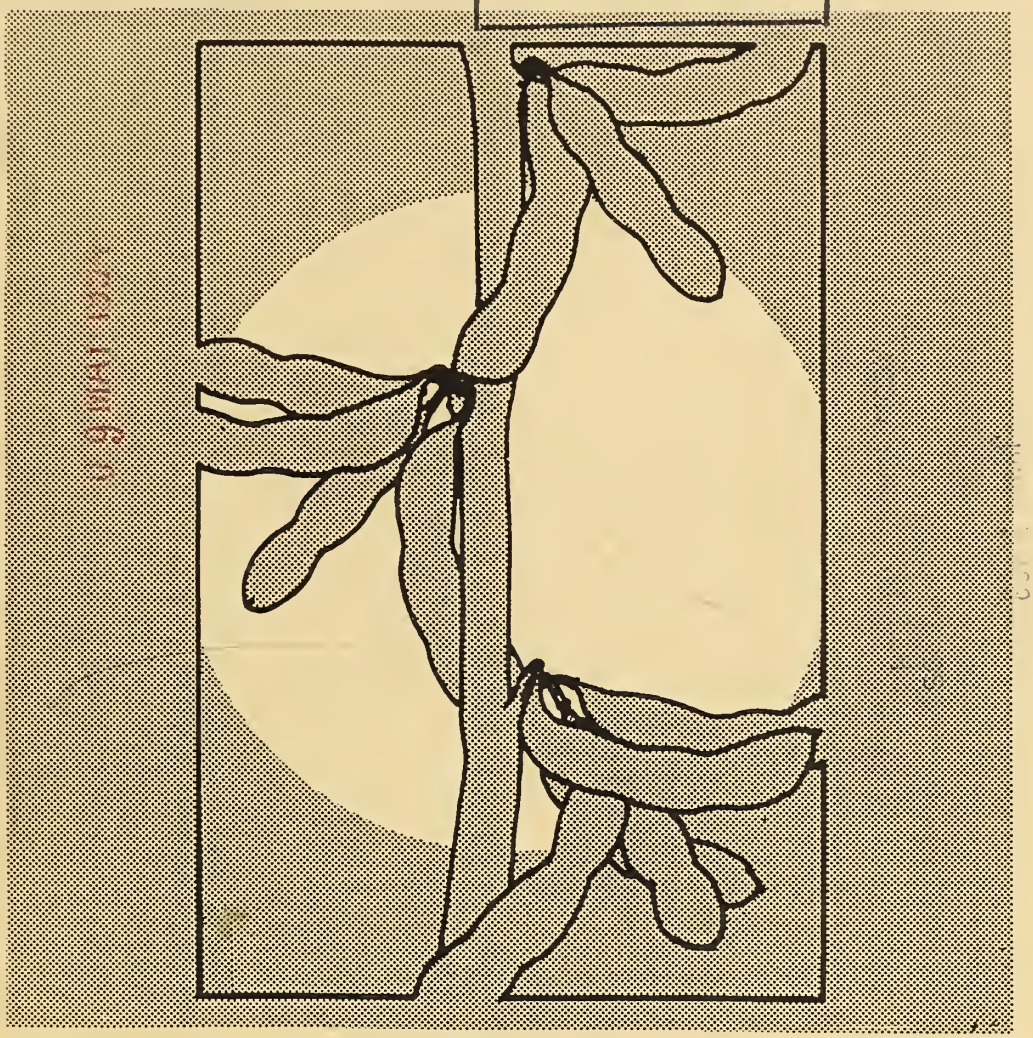
Soybean Genetics Newsletter



Volume 20 May 1993

The data presented here are not to be used in publications without the consent of the respective authors.

USDA-Agricultural Research Service
Department of Agronomy
and Department of Zoology / Genetics
Iowa State University
Ames, Iowa 50011

Table of Contents

Research Notes

Canada

China

Croatia

India

Slovakia

Thailand

United States

Foreword

In 1990, we found it necessary to institute a subscription fee for the Soybean Genetics Newsletter. We were naturally apprehensive concerning the response of readers and potential authors of news notes and articles to this charge. However, as you can see by the quantity and quality of Volume 20, our worries were unnecessary.

The response of soybean scientists all over the world to the Newsletter has been most gratifying to those of us responsible for publishing it. The worldwide derivation of the articles and the broad scope of themes - from breeding and pest resistance to chemical consititution and gene transfer - confirms our original belief, stated in Volume 1, 1974, that soybean researchers the world over needed a forum ". . . of an informal nature to stimulate thought and exchange ideas . . . preliminary in nature and speculative in content . . . ". We believe such reports can be exceedingly valuable and helpful, if viewed in the proper perspective.

The response of individuals, universities and institutions has been most gratifying as well. Especially appreciated is the financial assistance of several agri-business corporations who, in past years, helped fund the reprinting of several issues that had gone out of print but were still in constant demand. Their monetary help was also a psychological boost for us.

As we have said over these many years, the success of the Soybean Genetics Newsletter depends upon YOU, the soybean scientists, and we think that this volume, along with all the others, is reason for all of you to be proud.

Our sincere appreciation goes to those volunteers who made it all possible: Arricka Earp, Terry Couch, Marcia Imsande, Ellen Martens and Kim Lewers. These graduate students and technicians did a lot of the detail work that always accompanies any such project.

- R.G. Palmer, editor

SOYBEAN GENETICS COMMITTEE REPORT
February, 1993
Minutes of the Meeting

The Soybean Genetics Committee met from 7:00 to 9:00 p.m., February 22, 1993, at the Sheraton Plaza Hotel in St. Louis, Missouri, in conjunction with the Soybean Breeders Workshop.

Committee members attending the meeting were: G. R. Buss, P. B. Cregan, S. A. Mackenzie, R. L. Nelson, C. D. Nickell, R. C. Shoemaker, and J. E. Specht. H. Skorupksa and J. E. Specht had been elected by mail ballot to serve a three-year term on the Committee. At the conclusion of the meeting P. Cregan was elected Chair for the year ending in February, 1994.

Also in attendance at the meeting were T. Devine, C. Coble, J. R. Wilcox, E. Hartwig, R. L. Cooper and R. Leffel. Current Committee members and February expiration dates for their terms on the Committee are:

P. B. Cregan, USDA-ARS (1995) Chair
Nitrogen Fixation and Soybean
Genetics Laboratory
Bldg. 011, BARC West
Beltsville, Maryland 20705
(301)-504-5070
FAX (301)-504-5728

R. I. Buzzell (1994)
Crop Science Section
Agriculture Canada Research Station
Harrow, Ontario NOR 1GO, Canada
(519)-738-2251
FAX (519)-738-2929

R. L. Nelson, USDA-ARS (ex officio)
Turner Hall-Agronomy
1102 South Goodwin
University of Illinois
Urbana, Illinois 61801
(217)-244-4346
FAX (217)-333-4639

C. D. Nickell (1995)
Turner Hall-Agronomy
1102 South Goodwin
University of Illinois
Urbana, Illinois 61801
(217)-333-9461
FAX (217)-333-9817

R. G. Palmer, USDA-ARS (ex officio)
Department of Agronomy
Iowa State University
Ames, Iowa 50011
(515)-294-7378
FAX (515)-294-2299

R. C. Shoemaker, USDA-ARS (1994)
Department of Agronomy
Iowa State University
Ames, Iowa 50011
(515)-294-6233
FAX (515)-294-2299

H. T. Skorupska (1996)
Department of Agronomy and Soils
Clemson University
Clemson, South Carolina 29634
(803)-656-3523
FAX (803)-656-3443

J. E. Specht (1996)
Department of Agronomy
Keim Hall, E. Campus
University of Nebraska
Lincoln, Nebraska 68583
(402)-472-1536
FAX-(402)-472-7904

Procedure: As in the past, manuscripts concerning qualitative genetics interpretation, gene symbols, and linkages should be sent to the chairman of the Soybean Genetics Committee for review. To facilitate the review process, the Committee will proceed as follows:

1. The review will only be for "validity of the genetic interpretation" and "appropriateness of gene symbol." Manuscripts will not be reviewed for style except as this influences the clarity of interpretation. Manuscripts will not be "peer reviewed" unless requested by the author. Authors may submit unpublished (but comprehensible) manuscripts for review, unless peer review is requested. This should reduce delays involved in publishing a paper.

2. Reviewers of manuscripts will be given a deadline of three weeks to return the reviewed manuscript to the Chairman (who will then give it to the author as soon as possible). If the reviewers have not returned the manuscript by this time (or phoned in their comments), a phone call will be made to remedy the situation. If authors have not received a reply within two months of submission, they should contact the Chairman of the Soybean Genetics Committee.

Assignment/Approval of Gene Symbols: If gene symbols are being assigned in genetic studies where the material is from induced mutants, variants from heterogeneous populations, or from transgenic changes, then the authors should deposit representative genetic material in the Genetic Type Collection. Dr. R. L. Nelson is Curator for all maturity groups.

Gene symbols will be approved only in cases where the relevant material is available in one of the soybean germplasm collections for distribution to researchers. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made available. (Publication of genetic interpretation does not depend upon symbols, in most cases.) The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is not necessary.

Summaries for the Past Year: A list of soybean gene symbols and linkages approved during the year March 1992 through February 1993 is given to Table 1.

Committee Actions: A committee of R. C. Shoemaker (Chair), P. B. Cregan, and J. E. Specht presented a report on the 'Nomenclature of RFLP Loci' that was approved by the Soybean Genetics Committee.

Nomenclature of RFLP loci: The following guidelines are recommended for the nomenclature of probe-detected (RFLP) loci in soybean. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybeans.

1. Loci designations should be prefixed with a modified postal state identifier and/or institution identifier that will minimize ambiguity from similarities in probe names.

   e.g. Iowa State University --- IaSU
   University of Nebraska --- UNe
   University of Maryland --- UMd
   Indiana State University--- InSU
   U.S.D.A. Beltsville --- USDA

2. The prefix is followed by a string of letters and/or integers that identify the probe used to detect the locus by the originating laboratory. This probe-identifying string should be limited to more than six characters. This string should be separated from the prefix by a hyphen.

   e.g. IaSU-B317
   UNe-3012
   USDA-BLT27

3. Duplicate loci detected by the same probe should be identified with the same letter and integer base differentiated by integers (1, 2, 3, 4, etc.,) consecutively assigned in the order of publication. These numerals are to be separated from the base string by a hyphen.

   e.g. IaSU-B317-1
   IaSU-B317-2
   IaSU-B317-3

4. Upon publication of new RFLP loci researchers are strongly encouraged to

   a) make the probe identifying the locus/loci publicly available

   b) make available the identity of the restriction endonuclease used to generate the mapped polymorphism

   c) make available the identity of the genetic stock used to map the locus/loci

   d) make available the molecular weights of the polymorphic fragments used to map the locus/loci

5. These guidelines are also recommended for the nomenclature of loci identified through the application of DNA amplification techniques. When this applies, the sequence of the oligonucleotide or oligonucleotides used as primers in the amplification reaction should be made publicly available.

A committee of J. E. Specht (Chair) and R. L. Nelson was formed to investigate how possible future restrictions (through material transfer agreements) on genes that are approved by the Soybean Genetics Committee will affect future gene symbol assignments.

In the future the Soybean Genetics Committee will, upon assignment of a gene symbol, send the designation and request the authors to send a copy of the manuscript with information on the new genes to R. C. Shoemaker to be placed in Soybean Database. An updated list of gene symbols will be available from the database.

All future correspondence should be addressed to P. B. Cregan, Chair, Soybean Genetics Committee.

C. D. Nickell
Past Chair

Table 1. Gene symbols and linkage groups approved, March 1992– February, 1993.

Date	Authors	Trait/Linkage	Gene/Linkage	Genetic type
March 27,1992	Bowers, Ngelika, and Smith	Resistance to stem canker	*Rdc3, Rdc4*	
May 1, 1992	Kilen and Tyler	Linkage group 10 *Rps1*	*Rps1–27.0±4.3–L2*	
May 18, 1992	Pracht, Nickell, and Harper	Non–nodulation	*Rj5, Rj6*	
June 2,1992	Stephens, Nickell, and Kolb	Resistance to *Fusarium solani*	*Rfs*	
August 6, 1992	Yu and Kiang	Alcohol dehydrogenase Shikimate dehydrogenase Mannose–6–phosphate isomerase Phosphoglucomutase Linkage Group 21	*Adh3* *Sdh* *Mpi–e* *Pgm2–d* *Fle–9.8±1.3–Dia2*	
August 6, 1992	Pfeiffer, Hildebrand, and Orf	Lipoxygenase–1 allozyme	*Lx1–b*	
November 5, 1992	Yu and Kiang	Leaf margin necrosis in wild soybean	*Lmn*	

1993 SOYBEAN GERMPLASM CROP ADVISORY COMMITTEE REPORT

The Soybean Germplasm Crop Advisory Committee (SGCAC) held its annual meeting February 22, 1993, at the Soybean Breeders Workshop in St. Louis, Missouri. Twelve of the 14 members were in attendance. Members at the meeting were: W. Kenworthy, R. Nelson, J. Hicks, F. Schmitthenner, C. Coble, D. Egli, T. Kilen, L. Young, E. Hartwig, T. Devine, J. Thorne, and R. Boerma. Dr. P. Miller has retired from the USDA-ARS National Program Staff and since his position has not been filled a representative of the National Program Staff was not present on the committee. The current members of the committee and the dates of expirations of their terms are:

Name	Area of Representation	Expiration Date
H. Roger Boerma (706) 542-0927 FAX (706) 542-0914	Public Breeding South	1996
Claudia J. Coble (217) 244-4345 FAX (217) 333-4639	USDA Germplasm Collection	ex officio
Thomas E. Devine (301) 504-6375 FAX (301) 504-3260	Cytogenetics & Molecular Genetics	1994
Dennis B. Egli (606) 257-7317 FAX (606) 258-1952	Physiology	1995
Edgar E. Hartwig (601) 686-9311 FAX (601) 686-5465	USDA Germplasm Collection	ex officio
John D. Hicks, Jr. (601) 335-9152 FAX (601) 335-9164	Private Breeding South	1995
William J. Kenworthy (301) 405-1324 FAX (301) 314-9041	Public Breeding North	1994

Thomas C. Kilen (601) 686-9311 FAX (601) 686-5465	USDA Germplasm Collection	ex officio
Lavone Lambert (601) 686-5292	Entomology	1994
Vacant	USDA National Program Staff	ex officio
Randall Nelson (217) 244-4346 FAX (217) 333-4639	USDA Germplasm Collection	ex officio
A. Fritz Schmitthenner (216) 263-3847 FAX (216) 263-3841	Plant Pathology	1995
John Thorne (319) 653-6645 FAX (319) 653-3548	Private Breeding North	1996
Lawrence D. Young (901) 425-4741 FAX (901) 425-4729	Nematology	1996

Dick Bernard, University of Illinois and Yiwu Chen, Institute of Crop Germplasm Resources CAAS, Beijing, China, were present as observers. But to budgetary constraints, the invited observer from Canada could not be present.

Roger Boerma called the meeting to order and instructed the three Soybean CAC subcommittees of Acquisition, Evaluation and Operations to discuss issues and bring recommendations to the entire committee. Subcommittees were as follows:

Acquisition: W. J. Kenworthy;, chair, R. L. Nelson and J. D. Hicks; Evaluation: A. F. Schmitthenner, chair, H. R. Boerma, D. B. Egli, T. C. Kilen, and L. D. Young;

Operations: J. Thorne, chair, C. J. Coble, E. E. Hartwig, and T. E. Devine.

Subcommittee reports given

Acquisition Subcommittee

1. The subcommittee recommended that the originators of the several *rj1* isolines document the pedigrees of the isolines in a registration article or some referable document, such as the Soybean Genetics Newsletter, and place seed of the isolines in the soybean germplasm collection. The recommendation was adopted and endorsed by the SGCAC.

2. The subcommittee recommended that the 12 heterogeneous germplasm releases GP #13 to #17, GP #19, GP #33, GP #35 to #39 not be regrown at Urbana, Illinois, but be retained in the germplasm collection at Fort Collins, Colorado. The recommendation was adopted and endorsed by the SGCAC.

3. The subcommittee recommended that two years following requests for large numbers of accessions (100+) a letter be sent from the curator to the recipient to request evaluation data for inclusion in the GRIN database. The recommendation was adopted and endorsed by the SGCAC.

4. The subcommittee recommended that the developers of near-isogenic lines be encouraged to enter these lines in the GRIN system. The recommendation was adopted and endorsed by the SGCAC.

Evaluation Subcommittee

1. The National Program Staff guidelines for the use of ARS soybean germplasm evaluation funds called for an allocation of 26% for disease resistance evaluation, 26% for nematode resistance evaluation, and 48% for insect resistance evaluation. Since damage from insects is less severe and prospects for transfer of insect resistance genes by asexual transgenic means are good, the committee recommended that the allocation of funds should be altered to one-third each for insect problems, disease problems and nematode problems. The recommendation was adopted and endorsed by the SGCAC.

2. A Core Collection of soybean germplasm consisting of a sub-sample of the 13,000 accessions in the total collection would provide an expedient tool for sampling the collection for variability for specific traits without evaluating all 13,000 accessions

and would provide a more effective method of comparing the association of traits. The Core Collection would provide a previously agreed upon representative sample of the germplasm which could be supplied to fill most requests for the entire collection and thus reduce resources required to increase seedlots and process seed requests. The subcommittee recommended that a Core Collection of soybean germplasm be established and that a committee be established to advise the curator, Dr. R. Nelson, on the establishment of the Core Collection. The recommendation was adopted and endorsed by the SGCAC.

3. In response to the subcommittee recommendation, Chairman Boerma appointed a committee consisting of R. Nelson (chairman), W. Kenworthy, and T. Devine to establish a Core Collection. The recommendation was adopted and endorsed by the SGCAC.

4. L. D. Young plans to complete screening Maturity Groups V-X for *M. incognita* and *M. arenaria* and then to screen Maturity Groups I-IV for *M. incognita*. The subcommittee concurred with his plans.

5. L. D. Young has completed screening all introductions not already evaluated by S. Anand at Missouri for cyst nematode races 3, 5, and 14. At Stoneville, Mississippi approximately 1,000 PI's have been screened for resistance to stem canker using the toothpick inoculation method. Over 50% of the accessions had a high degree of resistance. In 1993, he plans to screen 432 additional Maturity Group V lines for resistance. The subcommittee concluded that adequate resistance to stem canker was available and identified. The subcommittee recommended that emphasis for future evaluation should be devoted to frogeye leaf spot resistance which is complicated by variability for pathogenicity among races of the pathogen and to sudden death syndrome, a disease about which little is known. The recommendation was adopted and endorsed by the SGCAC.

Operations Subcommittee

1. The justification for maintaining a collection of cultivars developed by commercial breeders was considered. Very few private cultivars have been submitted to the germplasm collection by private companies. It was agreed that John Thorne

would discuss the subject with the commercial soybean breeders. It was suggested that the curator select specific classical cultivars of special significance and request seed of these cultivars from the originators for entry into the germplasm collection.

2. The distribution of Plant Variety Protected (PVP) germplasm was discussed. It was agreed to continue the current policy of facilitating the distribution of PVP material with the consent of the patent holder as stated in the original submission.

3. It was agreed that the primary responsibility of the curator was to maintain and distribute seed of the accessions in the collection and descriptive information about the accessions. It was also agreed that information on the number of requests for specific accessions should be available, but that supplying information detailing the individual requests is beyond the scope and mission of the curator's mandate.

Chinese germplasm collection

Dr. Yiwu Chen reported on the status on the soybean germplasm collection in China. The collection contains a total of 17,000 accessions of *G. max* and 5,000 lines of *G. soja*. The *G. max* accessions were collected as follows: 2,000 from the Northeast area of China, 7,000 from the Huang Huai Hai River Valley area, 7,000 from the area south of the Yangtze River, and 1,000 foreign lines. About 10,000 lines have been analyzed for resistance to soybean mosaic virus and cyst nematode. Approximately 6,000 accessions have been screened for drought resistance, 3,000 for cold resistance and 3,000 for salt tolerance. About 8,000 accessions have been screened for protein, oil, and linolenic acid content. The program is now collecting an additional 5,000 lines of *G. max* and 2,000 lines of *G. soja*.

Canadian Representative on the SGCAC

For many years, the SGCAC has welcomed a Canadian representative to the committee in a nonofficial capacity. The committee, recognizing the geographic and ecotypic continuity of the North American landmass and the common agricultural challenges to soybean production in North America, agreed that it would be appropriate to extend a formal invitation to Canadian soybean researchers to participate in the activities of the committee through representation by an official

member of the SGCAC. It was moved that the SGCAC request Canadian soybean researchers to nominate an official representative to the SGCAC and empower Roger Boerma to make the appropriate and necessary changes in the by-laws of the committee to accommodate an official Canadian representative. The motion was adopted by the SGCAC.

USDA Soybean Germplasm Collection Report

The collection contains approximately 13,000 *G. max* strains. In 1992, 500 germplasm accessions were received from the Institute of Crop Germplasm Resources in Beijing, China as a result of an agreement between the USDA and the Chinese Ministry of Agriculture. This effort was supported financially by the Illinois Soybean Program Operating Board, the Iowa Agriculture and Home Economics Experiment Station, the Iowa Soybean Promotion Board, and the USDA-Agricultural Research Service. These 500 new accessions were primarily from Central China and thus are particularly valuable in extending the range of variability in the USDA Collection since approximately 85% of the Chinese accessions in the Collection had come from three provinces in Northeast China. Chen Yiwu, assistant professor in the Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences in Beijing, arrived in the U.S. in May to spend one year learning about the operation of USDA Soybean Germplasm Collection and doing germplasm research.

In 1992, a total of 10,470 seedlots were distributed from the USDA Soybean Germplasm Collection in response to 417 requests from 40 states and 40 foreign countries. Approximately 160 new *G. max* lines from the former USSR, China, Japan, and Taiwan were added to the collection and are now available for distribution.

GRIN Update

As of September, 1991, a new computer was purchased and delivered. The hardware consists of a Solbourne computer with 40 gigabytes of storage space and 512 megabytes of memory with 120 MIPS of processing power. The software includes a UNIX operating system and the Oracle relational database package.

Over the next two years the GRIN/DBMU will be involved with the design and software development phase of the new system. During the design phase, new sources of data will be considered including isozymes, RFLPs, gene mapping studies, and international databases. The current GRIN system on the PRIME will continue to operate as usual until the new system is released. The new system will be operational by June of 1994.

A PC version of GRIN has now been developed and is available for distribution upon request. A copy of all the data for a crop or group of crops can be loaded to a diskette along with a menu-driven software package. This can then be copied to and used on any PC which will eliminate communication costs. The GRIN public system on the PRIME can also continue to be accessed via commercial phone lines and Internet.

T. E. Devine, USDA, ARS, Beltsville, Maryland, was elected Chairman and J. Thorne, Northrup King Company, Washington, Iowa, was elected Vice-Chairman of the Soybean Germplasm Crop Advisory Committee.

There being no other business, the meeting was adjourned.

H. Roger Boerma, Chairman
Soybean Germplasm Crop Advisory Committee

USDA SOYBEAN GERMPLASM COLLECTION REPORT

February 1993

In 1992, a total of 10,470 seedlots were distributed from the USDA Soybean Germplasm Collection in response to 417 requests from 40 states and 30 foreign countries. There were 318 domestic seed orders for 7,153 seedlots and 62 foreign requests for 2,623 seedlots. Numerous publications were sent in response to 28 domestic and 7 foreign requests for information about the collection. Additionally, 697 accessions were sent to the National Seed Storage Laboratory at Ft. Collins, Colorado as back-up samples for the collection.

Of the approximately 13,000 *Glycine max* strains in the Collection in 1992, 196 were grown in 4-row plots in Stoneville and 1,614 were grown in 4-row plots in Urbana for seed replacement. Of the 656 pureline and comparison plant rows grown in 1992, 131 were grown at Stoneville and 525 were grown at Urbana. Approximately 160 new *G. max* lines were added to the collection and are now available for distribution, as is a listing of these accessions. These additions are from the Russia, China, Japan, and Taiwan. New *G. max* and *G. soja* accessions received to date that will be planted in 1993 include accessions from Argentina, China, Japan, Korea and the Russia. Included in this group are 79 accessions from the Institute of Crop Germplasm Resources in Beijing, China. These lines are from the list of 619 accessions released by IBPGR as being available from the Institute of Crop Germplasm Resources. We requested these lines in 1989.

Maturity group X accessions have been purelined in Puerto Rico. Single plants of all group X accessions were harvested during the winter of 91-92. In early May, we planted 813 plant rows in order to complete the purelining process in one year. Some of the accessions ripened as expected in August and September but others didn't ripen until late December. The seed set on the late maturing lines was extremely poor. There is no obvious correlation between maturity date with a September planting date and maturity date with a May planting date. Until we better understand the environmental requirements of this material, we will not attempt to grow groups IX or X in the summer in Puerto Rico. We did select 298 purelines to be added to the

collection. Because of the seed production problems last summer, seed increases from some accessions will be planted next winter and seed will not be available for those lines until 1994.

The 153 group IX accessions and 194 new introductions received in 1991 from the Malang Research Institute for Food crops, Indonesia, were planted in October 1992 in Puerto Rico. Also planted with this group were an additional 5 new Chinese accessions received in 1992 from W. J. Kaiser of the USDA/ARS Regional Plant Introduction Station, Washington State University. These lines were collected in different provinces of the People's Republic of China in 1990 by W. J. Kaiser and F. J. Muehlbauer. Single plants were harvested this month and will be planted in rows in the Fall of 1993 to complete the pureline process.

In 1992, 500 germplasm accessions were sent from the Institute of Crop Germplasm Resources in Beijing. This was the result of an agreement signed between the USDA and Chinese Ministry of Agriculture and supported financially by the Illinois Agricultural Experiment Station, the Illinois Soybean Program Operating Board, the Iowa Agriculture and Home Economics Experiment Station, the Iowa Soybean Promotion Board, and USDA-Agricultural Research Service. The seeds arrived in Urbana on May 7 and were planted on May 15. Heavy rain and very cold temperatures immediately after planting caused some stand problems. Plants were harvested from all accessions but seeds from nine accessions were planted in greenhouse this winter to provide additional plant rows for 1993. The request was for primitive cultivars and the phenotypes confirmed that that criterion was met. We also requested that we be able to grow them to maturity at Urbana and that these accessions not come from Heilongjiang, Jilin, and Liaoning provinces. Approximately 85% of the Chinese accessions in the Collection had come from these three provinces. Table 1 lists the origin of the 500 new accessions and the number of accessions previously obtained from these provinces. All new accessions did mature at Urbana but most were quite late. Maturity groups will not be assigned after the growing season in 1993 but based on 1992 data approximately 85% will be group IV or later, 15% will group III and only 1% in maturity group I. Chen Yiwu, an assistant professor in the Institute of Crop Germplasm Resources, Chinese Academy of

Agricultural Sciences in Beijing, arrived with the seed and is spending a year learning about the operation of the USDA Soybean Germplasm Collection and doing germplasm research.

An additional 153 new accessions were grown for the first time at Urbana and another 55 at Stoneville. These originated from the Japan, China, Taiwan, Korea, Vietnam and Russia. The following institutions donated germplasm to our collection this year: National Institute of Agrobiological Resources. Tsukuba, Ibaraki, Japan; Yamagata University, Yamagata, Japan; Queensland Department of Primary Industries, Gatton Research Station, Queensland, Australia; Asian Vegetable Research and Development Center, Taiwan; Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, Beijing, China; Agricultural University, Hanoi, Vietnam; Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany; Vavilov Institute, Leningrad, Russia; All-Russian Soybean Institute, Blagoveshchensk, Russia. Three cultivars of unknown origin were provided by Johnny's Selected Seeds, Albion, Maine, USA. The following people helped to obtain these new accessions, and their assistance is greatly appreciated: Bai Xin Xue, Chinese Academy of Agricultural Sciences; R. L. Bernard, University of Illinois; T. Carter, USDA-ARS, North Carolina State University; G. Li, Washington State University; P. Hanelt, Institute of Plant Genetics and Crop Plant Research; T. Hymowitz, University of Illinois; W. J. Kaiser, USDA-ARS, Washington State University; Dr. Kawaguchi, National Institute of Agrobiological Resources; Y. T. Kiang and Hongrun Yu, University of New Hampshire; J. Konovsky, East-West Seed Company, Inc.; S. M. Lim, University of Arkansas; R. McMahon, Queensland Department of Primary Industries, E. D. Nafziger, University of Illinois; R. L. Palmer, USDA-ARS, Iowa State University; V. I. Sichar, Odessa, Ukraine; Tong Diaxing, Institute of Crop Germplasm Resources; and S. Wyrostek, Johnny's Selected Seeds.

Sixty-seven plots of *Glycine soja* were grown in Stoneville and 36 plots were grown in Urbana for seed increase in 1992. Only one new line was added to the wild soybean collection this year. The accession is from China and was provided by the Heilongjiang Academy of Agricultural Sciences, Harbin, Heilongjiang China, with the assistance of M. Rangappa, Virginia State University. The current inventory of the

USDA Wild Soybean Germplasm Collection is 1035 accessions. Three new accessions of wild soybean were grown for the first time in 1991. All three were donated by R. Palmer, USDA-ARS, Iowa State University, who obtained them from V. I. Sichar, Odessa, Ukraine.

This year we began the general evaluation if 812 accessions of group VI at Stoneville. These lines will be evaluated again in 1993. These data will be summarized and published in a USDA Technical Bulletin in 1994. Evaluation data for PI490.765 through PI 507.573 (groups 000 to IV) was published in USDA Technical Bulletin 1802 in 1991. This publication was distributed in July, 1992 to approximately 400 researchers throughout the world. Included in this mailing were updated versions of non-published information about the soybean collection. Data from L. D. Young's soybean cyst nematode screening study, L. Lambert's soybean looper study and T. C. Eldon's Mexican bean beetle study have been added to the GRIN database. The new data format allows the methods used in each study to be linked with the results.

We have been working with the Database Management Unit in Beltsville, Maryland in the development of structure design and functions of the GRIN3 database. A version of GRIN that can be loaded on a personal computer is now available to any bonafide domestic or foreign user. This stand-alone program, PCGRIN, does not require telecommunications capabilities since it is installed on an individual's PC. Currently, the soybean version of PCGRIN requires about 20 MB of space on a hard drive. If access to the Internet exists, it is possible to download this information directly to a PC from the Internet system using FTP. To obtain additional information about PCGRIN or to obtain a copy, contact the Database Manager, USDA/ARS/PSI/GRIN/DBMU, Room 407, Building 003, BARC-West, 103000 Baltimore Avenue, Beltsville, Maryland, 20705-2350, USA; Phone: 301-504-5666; FAX: 301-504-5536.

Two used silica-gel dehumidifiers were obtained from the National Seed Storage Laboratory (NSSL) at Ft. Collins, Colorado when the new building was built for the Laboratory. These units have been installed on two cold rooms at Urbana. The NSSL recommends 25% relative humidity (RH) for long-term storage. With the addition of this equipment we can keep the collection and seeds waiting to be processed into

collection at 50° and 25% RH. We are hoping that this improvement will increase both the longevity and vigor of the seeds in the collection. Seeds samples have been placed in storage at both 25% and 45% RH. Germination and vigor will be measured on these samples during the next 15 years.

The USDA Perennial *Glycine* Germplasm Collection is maintained through a cooperative agreement with Ted Hymowitz, University of Illinois. The most recent report on that collection can be found in the 1992 issue of the Soybean Genetics Newsletter.

C. J. Coble and R. L. Nelson
USDA-Agricultural Research Service
University of Illinois
1102 S. Goodwin Avenue
Urbana, Illinois 61801

Executive Board of Commercial Soybean Breeders - 1993

Chairman
Keith Bilyeu
R.R. 2, Hwy 30 E.
Ames, Iowa 50010
515-232-0707
Fax = 515-232-8169

Vice - Chairman
Tom Floyd
Dekalb Plant Genetics
R.R. 3, Box 302
Bloomington, IL 61704
309-829-6640
Fax = 309-829-6707

Secretary
Craig Davis
Central Golden Harvest Research
P.O. Box 248 (UPS: RT.9)
Pekin, IL 61554
309-346-5544
Fax = 309-346-5904

Scott Frank
SRF, Inc.
115 North Perry
Mason City, IL 62664
217-482-3219
Fax = 217-482-3967

Tim Foley
Jacques Seed Company
R.R. 2, Box 84
Lincoln, IL 62656
217-735-4485
Fax = 217-732-1541

Terry Walker
Illinois Foundation Seeds
P.O. Box 722, U.S. 45 S.
Champaign, IL 61820
217-485-6420
Fax = 217-485-5223

AGRICULTURE CANADA
Research Station
Harrow, ON NOR 1GO Canada
and
IOWA STATE UNIVERSITY
Departments of Agronomy & Zoology/Genetics
Ames, IA 50011

Canada

245

Mapping the w4 locus in soybean

The w4 locus controls the biosynthesis of anthocyanin in soybean flowers and hypocotyls (Hartwig and Hinson, 1962), but the enzyme encoded by the locus is not known. A mutable allele at the w4 locus (w4-mutable or w4-m) was described by Groose *et al.* (1988). The w4-m allele has many features commonly associated with transposable elements. Knowing the map location of w4 and any linked, easily scorable markers would facilitate genetic studies of transposable elements at w4. The objective of this study was to map w4 by using populations that were segregating for morphological and biochemical markers.

Materials and methods: The soybean lines used in this study, Harosoy-k2, Harosoy-w4, PI 437.477B, and PI 437.293, originally were obtained from Dr. R. L. Bernard, USDA-ARS, Urbana, IL. The soybean lines have been maintained at Ames, IA. The Harosoy-w4 isoline was the source of the recessive allele for the w4 locus. All other lines had a wild-type allele at w4.

Crosses were made in Ames and F_1 plants were grown in Isabela, P.R. or Ames, IA. The F_2 seed was germinated at 28°C for 72 hours on germination paper. Samples for electrophoretic analyses were taken from the cotyledons (according to Cardy and Beversdorf, 1984a and b) and the seedlings were transplanted into a sandbench in the greenhouse. The phenotype of each seedling for the W4 locus was determined from the hypocotyl color of the F_2 plants approximately seven to ten days after transplanting. The W4 locus has a pleiotropic effect on both flower and hypocotyl color. Seedlings with green hypocotyls will have near-white flowers and seedlings with purple hypocotyls will

have purple flowers. Pubescence tip (sharp Pb -- vs. blunt pb pb) was determined on the first trifoliolate leaf of the seedlings grown in the sandbench (Ting, 1946).

To test for linkage between W4 and K2 (tan saddle on the seedcoat), F_2 plants were grown in the field. Because the seedcoat is maternal tissue, seed from the F(n) generation has a seedcoat that exhibits a F(n-1) genotype. Therefore, the F_2 phenotype of each plant was determined from the presence (k2k2) or absence (K2) of a saddle on the F_3 seed.

The starch gel electrophoresis procedures of Cardy and Beversdorf (1984a and b) were used for isozyme analyses. The isozyme markers Enp, MDH, Pgi2, and Pgm1 were resolved on 13% starch gels with the "B" buffer system. The Aco2, Aco3, Aco4, Ap, Dia1, Idh1, and Idh2 loci were resolved on 13% starch gels with the "D" buffer system (Cardy and Beversdorf, 1984a and b). Electrophoresis was carried out at a constant power of 8.5 W per gel for 5 and 6 hours for the "D" and "B" gels, respectively.

Linkage estimates and Chi-square values were computer calculated using the Linkage-1 program (Suiter *et al.*, 1983). The initial linkage calculations based on 276 F_2 individuals from the cross PI 437.193 X Harosoy-w4 indicated that Pgi2 and W4 were linked. An additional 425 F_2 individuals from the cross were evaluated for W4 and Pgi2 to confirm the linkage. No other markers were scored on the additional individuals.

To test for linkage to the characterized primary trisomics, Harosoy-w4 was crossed to Tri A, B, C, and D. Segregation ratios in the progeny of trisomic F_1 plants were tested against the segregation ratios in the progeny of disomic F_1 plants (Palmer, 1976). The trisomics A, B, and C are w1w1 W4W4. The expected disomic F_2 segregation ratio is 9:7. If the W4 locus was located on the extra chromosome of one of the trisomics, the F_2 segregation ratio adjusted for the relative transmission rates of n and n+1 gametes would be approximately 45:19 (3/4 X 15/16) (Palmer, 1976). One hundred twenty-five F_2 individuals are required to distinguish between the trisomic and disomic segregation ratios (Mather, 1951). Because Tri D is W1W1 W4W4, F_2 segregation ratios in the progeny of 41-chromosome F_1 plants would be sufficiently different from the expected 3:1 ratio in the progeny of disomic F_1 plants, if W4 is located on the extra chromosome. Based on the transmission rate of the n+1 gamete through the male and female gametes, 65 individuals were required to distinguish between disomic and trisomic segregation ratios (Mather,

1951).

Results and discussion: All isozyme loci segregated 1:2:1 as expected (data not shown). The genetic mechanisms producing the malate dehydrogenase (MDH) zymogram have not been characterized. The variants segregating in this study were MDH A and B (as designed by Cardy and Beversdorf, 1984a. The ratio of MDH A and B in the F_2 generation is 3:1 (author, unpublished data) and, in this study, the MDH segregated as expected. Each of the morphological markers exhibited the expected 3:1 segregation ratio in the F_2 generation.

Initially, 276 F_2 plants from the cross PI 437.293 X Harosoy-w4 were scored for two morphological markers and seven isozyme markers. A significant linkage Chi-square was present for the test of linkage for Pgi2 with W4. The recombination value was approximately 42%. Hanson (1959) reported that 392 individuals were required to establish a linkage of 40% with a 95% probability. An additional 425 individuals were analyzed for W4 and Pgi2. The final linkage estimate was 44.8 ± 2.2% (Table 1). To establish a linkage of 45% with 95% confidence, 1610 individuals are required (Hanson, 1959). Because sufficient F_2 seed was not available, additional individuals were not evaluated. If W4 is linked to Pgi2, as the data suggest, the linkage value is close to independence. Several different cross combinations are required to confirm linkage estimates that are close to 50.0 map units.

The other markers segregating in the cross of PI 437.293 X Harosoy-w4, Aco3, Aco4, Dia1, Idh2, and Pb, were inherited independently of W4 (Tables 2 and 3). In the cross of Harosoy-w4 X PI 437.477B, 191 individuals were analyzed for isozyme and morphological markers. Each of the six isozyme loci, Aco2, Ap, Dia1, Enp, Idh1, and Idh2 were inherited independently of W4 (Table 2).

Two morphological markers, Pb (pubescence tip) and K2 (tan saddle on a yellow seedcoat), were tested for linkage with W4. Two F_2 populations were segregating for Pb and W4 and, in each instance, the two loci were inherited independently (Table 3). Analysis of the F_2 data from the cross of Harosoy-w4 X Harosoy-k2 indicated the W4 and K2 were not linked (Table 3).

The W4 locus also was tested to determine if this locus was located on the extra chromosome in one of the primary trisomics A, B, C, or D. Because W1 and W4 are

segregating in the crosses of Harosoy-w4 X Tri A, B, and C, the expected disomic segregation ratio was 9:7. Both the F_2 progeny of disomic and trisomic plants had 9:7 ratios (Table 4). Therefore, W4 was not located on Tri A, B, or C. The W4 locus was not located on Tri D because a 3:1 F_2 segregation ratio was observed in the progeny of disomic and trisomic plants (Table 4).

References

Cardy, B. J., W. D. Beversdorf. 1984a. Identification of soybean cultivars using isoenzyme electrophoresis. Seed Sci. Technol. 12:943-954.

Cardy, B. J., W. D. Beversdorf. 1984b. A procedure for the starch gel electrophoretic detection of isyzymes of soybean (*Glycine max* (L.) Merr.) Dept. Crop Sci. Tech. Bull. 119/8401. University of Guelph, Ontario, Canada.

Groose, R. W., H. D. Weigelt, R. G. Palmer. 1988. Somatic analysis of an unstable mutation for anthocyanin pigmentation in soybean. J. Hered. 79:263-267.

Hanson, W. D. 1959. Minimum family sizes for the planning of genetic experiments. Agron. J. 51:711-715.

Hartwig, E. E., K. Hinson. 1962. Inheritance of flower color of soybeans. Crop Sci. 2:152-153.

Mather, K. 1951. The measurement of linkage in heredity. 2nd ed. Methuen and Co., Ltd., London.

Palmer, R. G. 1976. Chromosome transmission and morphology of three primary trisomics in soybean (*Glycine max*). Can. J. Genet. Cytol. 18:131-140.

Suiter, K. A., J. F. Wendel, J. S. Case. 1983. Linkage-1: a PASCAL computer program for the detection and analysis of genetic linkage. J. Hered. 74:203-204.

Ting, C. L. 1946. Genetic studies on the wild and cultivated soybeans. J. Am. Soc. Agron. 38:381-393.

B.R. Hedges
R.G. Palmer USDA-ARS

Table 1. Test of linkage of W4 with Pgi2 using F_2 populations derived from the cross PI 437.293 X Harosoy-w4

Pgi2	a/a	a/b	b/b	Statistics
W4	135	237	137	No. of individuals = 701
w4w4	57	105	30	$X^{2\,a} = 9.86$ $p^b = 0.01$
				$R \pm SE^c = 44.8 \pm 2.3$

[a] Chi-square was calculated assuming a 3:6:3:1:2:1 ratio. The df = 2
[b] [b]p = probability of a greater chi-square
[c] [c]R ± SE = recombination value ± standard error

Table 2. Tests of linkage between w4 and eight other loci using F_2 populations in soybean.

Locus		Classes						No.	X^2 [a]	p[b]
		aaW4	abW4	bbW4	aaw4w4	abw4w4	bbw4w4			
PI 437.477B X Harosoy-w4										
Aco2	a/a x b/b[c]	30	72	37	10	27	15	191	0.16	0.92
Ap	b/b x a/a	37	75	27	14	27	11	191	0.08	0.96
Dia1	a/a x b/b	32	68	39	9	28	15	191	0.76	0.68
Enp	a/a x b/b	32	67	40	9	26	17	191	0.80	0.67
Idh1	b/b x a/a	37	76	25	16	27	9	190	0.29	0.86
Idh2	b/b x a/a	33	66	39	15	22	15	190	0.62	0.73
PI 437.293 X Harosoy-w4										
Aco3	b/b x a/a	51	100	41	12	47	12	266	3.71	0.16
Aco4	c/c x a/a	57	91	42	14	43	17	264	3.54	0.17
Dia1	a/a x b/b	54	91	55	13	40	23	276	2.96	0.23
Idh2	a/a x b/b	49	96	55	26	32	18	276	2.62	0.27

[a] Chi-square tests of independence assuming a 3:6:3:1:2:1
[b] [b]p = probability of a greater chi-square
[c] The alleles at a locus are a, b, or c

Table 3. Tests of linkage of W4 with the morphological markers K2 and Pb using F_2 populations in soybean

Locus tested[d]	Parental line[e]	A_ [a] W4	aa_ W4	A_ w4w4	aa w4w4	No.	X^2 [b]	p[c]
Pb	PI 437.477B	157	43	58	16	274	0.00	0.98
Pb	PI 437.293	107	32	41	10	190	0.25	0.61
K2	Harosoy-k2	596	200	214	76	1086	0.13	0.72

[a] A_ and aa are the dominant and recessive classess respectively, of Pb and K2
[b] Chi-square tests of independence were calaculated assuming a 9:3:3:1 ratio
[c] p = probability of a greater chi-square
[d] Locus tested for linkage with W4
[e] Harosoy-w4 is pbpb. PI 437.477B and PI 437.293 are PbPb

Table 4. Tests of linkage of W4 with soybean primary trisomics A, B, C, and D

Trisomic	Chromo. number	A_	aa	No.	X^2 [a]
A	40	83	89	172	
	41	94	95	189	0.166
B	40	92	81	173	
	41	96	88	184	0.072
C	40	479	375	854	
	41	288	249	537	1.317
D	40	193	66	259	
	41	242	75	317	0.554

[a] Chi-square values were calculated by using the disomic ratio as the expected ratio rather than a 9:7 or 3:1 ratio. With 1 df, the critical chi-square value at 5% is 3.84

NANJING AGRICULTURAL UNIVERSITY
Soybean Research Institute
Nanjing, Jiangsu 210014
The People's Republic of China

China

245

A study on suitable sample size in the conservation of landrace of soybeans

In the conservation of soybean germplasm, it is important to find a suitable sample size for germplasm reproduction which can maintain the population, be genetically stable, and not spend too much manpower and material resources. Unfortunately, so far, determining of the sample size in the conservation of crop germplasm was basically experiential rather than experimental. The present paper is trying to figure it out based on both experimental data and computer simulation.

Materials and methods: Mixtures of two soybean cultivars were used to imitate soybean landraces composed of two homozygous genotypes. Four pairs of cultivars (Suxie-1 and Nannong 87C-37, and Nannong 88-1 and Nannong 87C-39 in 1990, Nannong 88-1 and Nannong 87C-39, and Nannong 86-62 and Nannong 88C-19 in 1991) were used in field experiments. Among the cultivars, Nannong 87C-37, Nannonc 87C-39 and Nannong 88C-19 were green seeded and used as the marker genotype. Five populations with frequencies of marker genotype 0.5, 0.3, 0.1, 0.05, and 0.01, respectively for each of cultivar pairs were formed. In 1990, each population was planted in 68 rows of 3.33m in length and 0.5m apart in Jiangpu Experimental Station of Nanjing Agricultural University (NAU) on June 18, and seedlings were thinned two times after emergence. In 1991, each population was planted in 70 rows of 3.33m in length and 0.5m apart with 66 seeds per row in Weigang Experimental Station of NAU on July 13-14.

The Monte Carlo computer simulation was carried out to confirm the results from the field experiments. The factors and levels involved were as follows: five levels of marker genotype frequency (0.5, 0.3, 0.1, 0.05, and 0.01), two levels of field emergence rate (0.3 and 0.5), three levels of number of seeds per plant (100, 50 and 30), two levels of the standard deviation of number of seeds per plant (15 and 5), and ten levels of sample size (66, 132, 198, ..., and 660).

Three kinds of statistics, i.e. the curve of standard deviation, the lower boundary of 95% confidence limit, and frequency of distinction of a minor genotype, were used to determine what was a suitable sample size.

Results: The results from both field experiments and computer simulation showed:

(1) The suitable sample size varied with the frequency of marker genotype, and a sample size with 250-330 seeds for planting, and 150-200 plants in the field could fit most of the situation of the tested frequencies of marker genotype except the frequency of rare genotype was too small, for example 0.01 or less (Table 1 and 2).

Table 1. The standard deviation of marker genotype frequency (MGF) in germplasm population during various stages

Cultivar group	MGF	At planting (AP)				In growing (IG)				After harvesting (AH)			
		66	264	330	660	66	264	330	660	66	264	330	660
Nannong	.50	.0696	.0355	.0310	.0214	.0963	.0494	.0410	.0293	.0875	.0482	.0410	.0291
88-1	.30	.0534	.0272	.0244	.0172	.0572	.0295	.0262	.0189	.0662	.0391	.0342	.0247
&	.10	.0339	.0175	.0157	.0111	.0485	.0225	.0208	.0145	.0638	.0308	.0280	.0196
Nannong	.05	.0222	.0111	.0094	.0067	.0264	.0131	.0112	.0078	.0365	.0177	.0155	.0112
87C-39	.01	.0102	.0049	.0045	.0031	.0132	.0064	.0060	.0042	.0132	.0070	.0067	.0048
Nannong	.50	.0560	.0280	.0245	.0174	.0772	.0394	.0354	.0245	.0816	.0422	.0401	.0273
86-62	.30	.0542	.0262	.0234	.0165	.0719	.0335	.0302	.0202	.0802	.0366	.0327	.0231
&	.10	.0318	.0161	.0140	.0096	.0458	.0226	.0208	.0142	.0417	.0209	.0186	.0134
Nannong	.05	.0239	.0120	.0107	.0074	.0307	.0157	.0142	.0098	.0262	.0129	.0109	.0078
88C-19	.01	.0111	.0057	.0052	.0035	.0155	.0078	.0069	.0048	.0151	.0076	.0069	.0046
Simulated	.50	.0632	.0319	.0276	.0195	.0875	.0446	.0385	.0271	.0907	.0472	.0396	.0284
population	.30	.0558	.0277	.0257	.0183	.0793	.0409	.0363	.0249	.0821	.0426	.0380	.0262
	.10	.0379	.0189	.0159	.0107	.0533	.0266	.0219	.0155	.0554	.0279	.0229	.0163
	.05	.0266	.0137	.0120	.0081	.0378	.0193	.0164	.0117	.0393	.0202	.0171	.0124
	.01	.0122	.0066	.0053	.0039	.0174	.0084	.0077	.0056	.0183	.0088	.0081	.0058

Table 2. The disappearing frequency of marker genotype (DFMG) in germplasm population during various stages

Sample size	Nannong88-1 & Nannong87C-39				Nannong86-62 & Nannong88C-19					Simulated population			
	AP		IG		AP		IG			AP		IG	
	.0500	.0099	.0500	.0099	.0500	.0100	.1000	.0500	.0100	.0500	.0100	.0500	.0100
66	.023	.432	.095	.718	.047	.457	.014	.295	.711	.033	.510	.200	.725
132	.001	.202	.009	.517	.003	.202	.001	.071	.504	.001	.270	.037	.510
198	.000	.111	.002	.399	.000	.098	.000	.019	.372	.000	.135	.005	.371
264	.000	.029	.000	.270	.000	.044	.000	.003	.248	.000	.060	.000	.281
330	.000	.016	.000	.219	.000	.024	.000	.001	.174	.000	.039	.000	.192
596	.000	.000	.000	.067	.000	.001	.000	.000	.055	.000	.002	.000	.053
660	.000	.000	.000	.042	.000	.000	.000	.000	.024	.000	.001	.000	.029

Note: The DFMGs for those marker genotype frequencies not in the table were all zero.

(2) The difference of field emergence between genotypes caused the change of marker genotype frequency in field, and the difference of reproductivity between genotypes caused variation of marker genotype frequency after harvesting.

(3) The variation of marker genotype frequency and the frequency of distinction of a marker genotype increased with the advance of generation (Table 3 to 4).

Table 3. The standard error of genotype frequency (GF) in soybean landrace population with various GF after N generations

Generation	Nannong 88-1 & Nannong 87C-39					Nannong 86-62 & Nannong 88C-19				
	.500	.300	.100	.050	.010	.500	.300	.100	.050	.010
1	.04820	.03910	.03080	.01770	.00700	.04220	.03660	.02090	.01290	.00760
5	.10778	.08743	.06887	.03958	.01565	.09436	.08184	.04673	.02885	.01699
10	.15242	.12365	.09740	.05597	.02214	.13345	.11574	.06609	.04079	.02403
15	.18668	.15143	.11929	.06855	.02711	.16344	.14175	.08095	.04996	.02943
20	.21556	.17486	.13774	.07916	.03130	.18872	.16368	.09347	.05769	.03399

Table 4. The DFMG in the simulation population with various GF after N generations

Generation	.01	.05	.10	.30	.50
1	.264	.002	.000	.000	.000
5	.635	.128	.025	.000	.000
10	.753	.342	.115	.001	.000
15	.832	.463	.227	.004	.000
20	.880	.560	.302	.024	.000

(4) In addition, the purity of a germplasm was disturbed due to the introduction of off-type, and which was getting more serious with the advance of generation (Table 5).

Table 5. The migration frequency in a population after N migration generation

Generation	0.01		0.05	
	Frequency	Standard error	Frequency	Standard error
1	.019805	.000394	.099127	.000843
5	.060120	.001257	.294432	.002419
10	.111058	.002263	.540939	.003956
15	.161758	.003267	.787735	.004645
20	.210283	.004005	.951505	.002915

References

Gao Kai etc. 1990. Study on the population size in seed reproduction of corn variety. Journal of Crops (China) (4):314-320.

Ma Yuha. 1982. Experimental Statistics. Agriculture Publishing House. Beijing, China.

Brown, A.H.D. 1978. Isozymes plant population genetic structure and genetic conservation. Theor. Appl. Genet. 52:145-157.

Crossa, J. 1989. Methodologies for estimating the sample size required for genetic conservation of outbreeding crops. Theor. Appl. Genet. 77:153-161.

Frankel, O.H. & E. Bennett. 1970. Genetic Resource In Plants: Their Exploration And Conservation. Blackwall, Oxford.

Frankel, O.H. & Hawkes, J.G. 1975. Crop Genetic Resource for today and tomorrow. Cambridge Univ. Press.

Fraser, A.S. 1957. Simulation of genetic system by automatic digital coputers. Austranlin J. Biol. Sci. 10:484-491.

Yonezawa, K. 1985. A definition of optional allocation of effect in conservation of plant genetic resource with application to sample size determination for yield collect. Euphytica 34:345-354.

Gai Junyi
Zhang Yuanming

SOYBEAN INSTITUTE
Jilin Academy of Agricultural Sciences
Gongzhuling, 136100
Jilin Province
The People's Republic of China

245

Comparison analysis of lipid peroxidation of soybean with different resistant level to *Leguminivora glycinivorrella* (Mats.)

Leguminivora glycinivorrella (Mats.) is a main insect pest in major soybean production areas. It causes the decrease of the output and quality of soybeans. There are few studies on Leguminivora glycinivorrella, especially the studies on resistant mechanisms. Most of the studies are focused on the screening and identification of resistant genotypes. There is no report about physiological and biochemical mechanisms of resistance of soybean to Leguminivora glycinivorrella. The purpose of this study was to analyze the resistant mechanisms of soybean from several aspects, so as to provide theoretical accordings of resistance of soybean to L. glycinivorrella.

Materials and methods: The resistant an susceptible genotypes to L. glycinivorrella were defined by identification for several years. Six resistant genotypes, including Huinan Heitiejia, Huaide Maoyandou, Huinan Ludadou, Gongjiao 5205, Jilin 16 and Maojiahuang, and three susceptible genotypes, including Pingdingxiang, Heiqizi, and Jilin 27 were used in this study. They are randomly planted in the field on the 4th of May at the form of one genotype in one line, and repeated for four times, covered with nylon net on the 10th of August. We placed the mature insects into the net at the pod filling period. Ten pods developed consistently and were taken for biochemical analysis when the pods were injured by insects most seriously.

Assay of superoxide dismutase (SOD) followed the method of Shao et al. (1983). Determination of peroxidation (POD) followed the method of Mead (1976). Assay of Catalyse (CAT) referred to the method of Shao (1983). Determination of chlorophyll referred to the Handbook of Plant Physiology (1980, 1987).

Results: 1) Result in table 1 showed that: SOD activity in the pod of the resistant genotypes (except Maojiahuang) was higher than that of susceptible genotypes, but there

was no clear trend in the seeds. 2) It can be seen in Tables 2 and 3 that: there are great differences for POD and CAT activities among different genotypes, but there is no evident regularity between the resistant and susceptible genotypes. 3) Determination of chlorophyll content was similar to the result of POD and CAT (Table 4).

Table 1. SOD activities of soybean with different resistant level

Name of cultivars	Resistant level	SOD activity (Units/g fr.wt) Pod shell	Seed
Huinan Heitiejia	R	1800	850
Huaide Maoyandou	R	1100	1900
Maojiahuang	R	550	1150
Huinan Ludadou	R	1650	1750
Gongjiao 5205	R	1450	1200
Jilin 16	R	1050	450
Pingdingxiang	S	500	1500
Heiqizi	S	550	1650
Jilin 27	S	900	1150

Table 2. POD activities of soybean with different resistant level

Name of cultivars	Resistant level	POD activity (Units/min gtf.) Pod shell	Seed
Huinan Heitiejia	R	223	101
Huaide Maoyandou	R	324	486
Maojiahuang	R	360	81
Huinan Ludadou	R	322	117
Gongjiao 5205	R	416	108
Jilin 16	R	153	390
Pingdingxiang	S	168	110
Heiqizi	S	360	258
Jilin 27	S	245	82

Table 3. CAT activities of soybean with different resistant level

Name of cultivars	Resistant level	CAT activity (Units/gft.) Pod shell	Seed
Huinan Heitiejia	R	4.08	6.80
Huaide Maoyandou	R	2.72	8.17
Maojiahuang	R	4.08	9.52
Huinan Ludadou	R	16.32	8.16
Gongjiao 5202	R	2.72	1.36
Jilin 16	R	10.88	4.09
Pingdingxiang	S	2.72	2.72
Heiqizi	S	10.88	4.08
Jilin 27	S	5.44	5.44

Table 4. Comparison of chlorophyll content of soybean with different resistant level

Name of cultivars	Resistant level	Chorophyll content (g/L) Pod shell	Seed
Huinan Heitiejia	R	394	322
Huaide Maoyandou	R	833	460
Maojiahuang	R	659	1221
Huinan Ludadou	R	1217	1231
Gongjiao 5205	R	312	674
Jilin 16	R	230	200
Pingdingxiang	S	266	383
Heiqizi	S	552	284
Jilin 27	S	598	241

Discussion: We can see from the results described here that the activities of POD and CAT were not related to the resistant level of soybean to L. glycinivorrella. It seems that there were no relationships between these biochemical factors and resistant level. Further experiments are needed to search for the reasons related to the L. glycinivorrella resistance. It should be noted that sample-taking is very important in this study, but it is difficult to assure the samples to be at the same development period, although we tried our best to make the samples identical.

References:

Huadong Normal Univ. 1980. Handbook of plant physiology. People's Education Press. pp:210-213.

Mead, J.F. 1976. In "Free radicals in biology". Academic Press. NY pp:51-68.

Shao Congben. 1983. Plant physiology communications. 5:46-49.

Yao Derong et al. 1987. Jilin agricultural sciences. 1987 (1): 40-41.

Zuang Bingchang
Wang Yumin
Xie Xueju
Xu Bao
Yao Derong
Li Jianping

JILIN ACADEMY OF AGRICULTURAL SCIENCES
Soybean Research Institute
Gongzhuling, Jilin Province 136100
The People's Republic of China

245

A preliminary study on protein and oil contents of wild soybean (*G. soja*) in Jilin province*

The protein and oil contents of 674 genotypes of wild soybean (G. soja) were determined and the relationship between protein and oil contents and some agronomic characters were analyzed in this experiment. Some special genotypes with high protein oil content were screened out.

Materials and methods: All of the genotypes used in this experiment were from Jilin province. The characters determined in this experiment include protein and oil contents, hilum color, seed coat color, weight of 100 seeds, leaf form, growing period and plant height. Some statistical parameters were listed in Table 1.

Table 1. Mean performance of characters of wild soybean

Characters	X ± Sx	Range	Coefficient of variation	
			%	Order
Protein content (%)	48.5 ± 2.6	37.3 - 55.4	5.36	5
Oil content (%)	8.3 ± 1.2	4.8 - 13.9	14.32	3
Protein add oil amount content (%)	56.9 ± 2.6	46.7 - 63.2	4.65	6
Plant height (m)	2.21 ± 0.48	0.68 - 3.40	21.72	2
Weight of 100 seeds (g)	1.6 ± 0.36	0.5 - 3.0	22.69	1
Growing period (day)	127.1 ± 9.1	90 - 139	7.19	4

Results and discussion: Dates in Table 2 showed that, mean content of protein is 48.5-2.6% and the highest content is 55.4%. The protein content of 204 genotypes are over 50%. Mean content of oil is 8.3 - 1.2% and the highest content is 13.9%. The oil content of 48 genotypes is over 10%.

*This project is supported by NNSF of China

Mean content of protein add oil amount content is 56.9 - 2.6% and the highest content is 63.2%. The protein add oil amount content of 75 genotypes are over 60%.

Table 2. Range of protein, oil, protein add oil amount content and their frequency of distribution

Proteins	Range	≤ 42%	42.1-46%	46.1-50%	50.1-54%	≥ 54.1%
	Number	2	100	368	201	3
	Percent to amount %	0.30	14.84	54.60	29.82	0.44
Oil	Range	≤ 6%	6.1-8%	8.1-10%	10.1-12%	≥ 12.1%
	Number	13	241	372	43	5
	Percent to amount %	1.93	35.76	55.19	6.38	0.74
Protein add oil amount	Range	≤ 50%	50.1-54%	54.1-58%	58.1-62%	≥ 62.1%
	Number	10	129	294	228	13
	Percent to amount %	1.48	19.14	43.62	33.83	1.93

The results described above showed that the content of protein in wild soybean with black-brown hilum, black seedcoat, ovete leaf and weight of 100 seeds is 1.6-2.5 g higher. The content of oil in wild soybean with black hilum, two-color seedcoat, ovete leaf and 2.6 - 3.0 g/100 seeds is higher. There is highly significant negative correlation between protein content and oil content.

Table 3. Comparison of protein and oil content of wild soybean with different agronomic characters

Characters	Types	Number	Protein content		Oil content	
			%	CV.	%	CV.
Hilum color	Black-brown	29	49.3a*	4.89	8.4ab	11.96
	Brown	495	48.6a	5.20	8.2b	17.26
	Black	100	48.0b	5.83	8.4a	14.05
Seedcoat color	Black	647	48.7a	5.08	8.4b	13.61
	Brown	12	48.2a	6.11	8.6ab	25.04
	Two-color	15	48.2a	8.07	9.3a	26.03
Leaf form	Ovete	352	48.8a	5.08	8.5a	13.48
	Long-ovete	264	48.5a	5.43	8.4a	14.64
	Line form	6	48.4ab	3.72	7.7b	14.90
	Lanceolate	52	47.6b	5.59	7.8b	15.43
Weigh of 100 seeds	2.1 - 2.5 g	51	49.0a	5.07	8.9b	13.78
	1.6 - 2.0 g	251	48.9a	2.04	8.5c	11.88
	1.1 - 1.5 g	304	48.5a	5.37	8.2d	13.81
	1.0 g ≥	48	47.5b	4.97	8.1d	17.02
	2.6 - 3.0 g	23	42.5c	6.95	12.8a	8.13

* The means within characters followed by the same letter are not significant at the 0.05 probability.

Eight special genotypes with both high protein and oil content were screened out from 674 genotypes of wild soybean in Jilin province. All these germplasm have black hilum and black seedcoat. Performance of eight genotypes of wild soybean were listed in Table 4.

Table 4. Performance of characters of special wild soybean germplasm

Entry number	Protein content (%)	Oil content (%)	Protein add oil amount content (%)	Growing period (day)	Weight of 100 seeds (G)	Plant height (m)
1	51.23	11.03	62.26	132	2.5	2.35
2	51.59	10.09	61.68	133	1.7	3.00
3	53.78	9.04	62.82	124	1.5	2.90
4	53.34	9.20	62.54	127	2.0	1.75
5	53.60	9.55	63.15	129	1.7	2.50
6	53.49	9.71	63.20	126	1.5	1.80
7	53.34	9.50	62.84	124	2.0	2.15
8	53.52	9.02	62.54	123	1.1	2.82

References

Xu Bao et al. 1984. Soybean Science 3(4):327-331.
Zheng Huiyu et al. 1980. Sci Agri. Sinica (4)22-26.
Yang Guangyu et al. 1986. Soybean Science 5(2):175-180.
Li Fushan et al. 1986. Soybean Science 5(1):65-72.
Hymowitz, T. et al. 1972. Agron. 63(5):613-616.
Rubel, A. et al. 1972. Crop Sci. 14(5):713-715.

Yang Guangyu
Zheng Huiyu
Han Chunfeng
Ji Feng

SOYBEAN RESEARCH INSTITUTE OF OF NANJING AGRICULTURAL UNIVERSITY
Nanjing, Jiangsu 210014
The People's Republic of China

A study on pod-bearing habit and related component characters in soybeans

Stem termination, or pod-bearing habit, is a morphologically and ecologically important trait for soybean breeding. Traditionally, it is classified into three types: determinate, semi-determinate and indeterminate, based on performance of a series of traits such as length of flowering period, growth habit, variation of stem diameter from bottom to top, number of nodes on main stem, variation of leaf size from bottom to top, pod set on terminal raceme, etc. Bernard's method has been widely accepted. However, it is still difficult in practice to clarify the pod-bearing habit of some soybean varieties by using Bernard's method, and the results of identification may vary among individuals.

Materials and methods: In order to find a simple and clear method in classifying types of pod-bearing habit of soybeans in field, 976 landraces from Shandong, Anhui, Jiangsu, Jiangxi and Fujian provinces, and F2 and F3 population of N6582 X HanJiangDaHuaLian (Dt X Si), and F3 population of JiangYinBiLueQing X N6582 (In X Dt), were studied in Nanjing in 1988-1990. The traits involved were as follows: Bernard's types of stem termination, number of nodes on main stem (NNO), number of nodes below the node with the largest leaf on main stem, existence of terminal inflorescence on main stem (ETIMS), length of terminal inflorescence on main stem, number of flowers on terminal inflorescence on main stem, leaf width and leaf length of the top one, leaf width and leaf length of the largest one, number of nodes above the node with the largest leaf on main stem (NNA), relative value of number of nodes above the nodes with the largest leaf of main stem (RVNN, i.e. NNA/NNO), ratio of leaf width of the top one to that of the largest one (RLWTL).

Results: The results were as follows:

1. RVNN, RLWTL and ETIMS were found to be the major component characters of pod-bearing habit.

2. Pod-bearing habit could be divided relatively and clearly into determinate, semi-determinate and indeterminate by using the major component characters, either ETIMS + RVNN or ETIMS + RLWTL (Table 1 to 4).

3. The criteria of pod-bearing habit types were:

Determinate type: with obvious ETIMS
RVNN<0.2, RLWTL≥0.7

Semi-determinate type: with obvious ETIMS
RVNN≥0.2, RLWTL<0.7

Indeterminate type: without obvious ETIMS
RVNN≥0.2, RLWTL<0.7

4. There was a coincidence between the major component method and Bernard's method (Table 5 to 6). The conclusion was further demonstrated with the data of another set of landraces in 1992 (Table 7). Since the major component method is simple and clear in field classification, it is suggested that the major component method may substitute for Bernard's method in field evaluation.

Table 1. Frequency distribution of the relative of number of nodes above the node with the largest leaf on main stem (RVNN)

Origin	Pod-bearing habit	0.0-	0.1-	0.2-	0.3-	0.4-	0.5-	0.6-
Shandong	Dt	118	20	6				
	Si		3	51	84	83	21	1
	In				8	53	23	5
Jiangsu and Anhui	Dt	54	19	5				
	Si			22	63	84	23	2
	In				8	18	34	4
Jiangxi and Fujian	Dt	21	3					
	Si		1	37	48	35	4	
	In				2	7	5	1
Total of each type	Dt	193	42	11				
	Si		4	110	195	202	48	3
	In				18	78	62	10
Grand total		193	46	121	213	280	110	13

Note: Dt=determinate, Si=semi-determinate, In=indeterminate. The same is true for the latter tables.

Table 2. Frequency distribution of ratio of leaf width of the top one to that of the largest on (RLWTL)

Origin	Pod-bearing habit	0.0-	0.1-	0.2-	0.3-	0.4-	0.5-	0.6-	0.7-	0.8-	0.9-
Shandong	Dt							8	5	43	88
	Si	6	37	35	35	49	47	25	9		
	In	20	36	22	6	5					
Jiangsu and Anhui	Dt							2	2	21	53
	Si	8	29	34	29	32	34	24	4		
	In	10	29	19	16						
Jiangxi and Fujian	Dt							3	1	8	12
	Si	3	13	17	25	30	17	16	4		
	In	1	3	8	3						
Total of each type	Dt							13	8	72	153
	Si	17	79	86	89	111	98	65	17		
	In	31	68	49	15	5					
Grand total		48	147	135	104	116	98	78	25	72	153

Table 3. Frequency distribution of the relative value of number of nodes above the node with the largest leaf on main stem (RVNN) in F2 and F3.

Cross Generation		Pod-bearing habit	0.1-	0.1-	0.2-	0.3-	0.4-	0.5-	0.6-
N6582 x HanJiang-DaHuaLian	F2	Dt	14	1	1				
		Si		2	10	18	44	4	4
		In							
		Total	14	3	11	18	44	4	4
	F3	Dt	53	3	1				
		Si		3	11	16	54	34	5
		In							
		Total	53	6	12	16	54	34	5
JiangYin-BiLuoQing x N6582	F3	Dt	51	4	3				
		Si		4	18	20	25	2	1
		In				6	21	28	4
		Total	51	8	21	26	46	30	5

Table 4. Frequency distribution of ratio of leaf width of the top one to that of the largest one (RLWTL) in F2 and F3.

Cross generation		Pod-bearing habit	0.0-	0.1-	0.2-	0.3-	0.4-	0.5-	0.6-	0.7-	0.8-	0.9-
N6582 x Han-jiang-Da-Hua-Lian	F2	Dt							1	1	2	12
		Si	2	3	5	15	14	27	13	3		
		In										
		Total	2	3	5	15	14	27	14	4	2	12
	F3	Dt							2	5	14	36
		SI	1	2	7	5	40	49	15	4		
		In										
		Total	1	2	7	5	40	49	17	9	14	36
Jiang YinBi Luo Qing x N6582	F3	Dt							4	5	16	33
		Si	1	3	14	25	12	9	3	6		
		In	4	20	28	6	1					
		Total	5	23	42	31	13	9	7	11	16	33

Table 5. Comparison of resultss classified by different methods of pod-bearing habit.

Method	Variety or cross	ETIMS + RLWTL			Bernard bernard's		
		Dt	Si	In	Dt	Si	In
	Variety	239(11)	545(11)	168(0)	235(15)	558(15)	168(0)
ETIMS + RVNN	N6582 X HanJiangDaHuaLian F_2	15(4)	79(4)		15(3)	80(3)	
	N6582 X HanJiangDaHuaLian F_3	59(3)	118(3)		56(4)	120(4)	
	JiangYinBiLueQing X N6582 F_3	60(4)	65(4)	59(0)	55(8)	66(8)	59(0)
	Variety				233(30)	545(30)	168(0)
EITMS + RLWTL	N6582 X HanJiangDaHuaLian F_2				15(4)	79(4)	
	N6582 X HanJiangDaHuaLian F_3				55(6)	119(6)	
	JiangYinBiLueQing X N6582 F_3				54(10)	65(10)	59(0)

Note: Figures not in parentheses are frequencies consistant for the two methods, and those in parentheses are not consistant ones.

Table 6. X^2 test of relation between methods classifying pod-bearing habit.

Major component character	Variety of cross	Type	Bernard's method		
			Dt	Si	In
ETIMS + RVNN	Variety	Dt	235	4	
		Si	11	558	
	$X^2 = 1871.71$	In			168
	N6582 F^2 X HanJiangDaHuaLian	Dt	15	2	
		Si	1	80	
	$X^2 = 77.85$	In			
	F^3	Dt	56	3	
		Si	1	120	
	$X^2 = 162.26$	In			
	JiangYinDaLuoQing X F^3 N6582	Dt	55	5	
		Si	3	66	
	$X^2 = 332.10$	In			59
ETIMS + RLWTL	Variety	Dt	233	17	
		Si	13	545	
	$X^2 = 1789.58$	In			168
	N6582 X F^2 HanJiangDaHuaLian	Dt	15	3	
		Si	1	79	
	$X^2 = 72.47$	In			
	F^3	Dt	55	4	
		Si	2	119	
	$X^2 = 153.68$	In			
	JiangYinBiLuoQing X N6582	Dt	54	6	
		Si	4	65	
	$X^2 = 332.01$	In			59

Note: $X^2_{0.01,1} = 9.21$ $X^2_{0.01,4} = 13.28$

Table 7. A further demonstration of coincidence between the two methods.

Major component character	Type	Bernard's method		
		Dt	Si	In
ETIMS + RVNN	Dt	26	4	
	Si		30	
	In			17
	$X^2=141.0$			
ETIMS + RLWTL	Dt	30		
	Si		30	
	In			17
	$X^2=152.9$			

References

Bernard, R.L. 1972. Two genes affecting stem termination in soybean. Crop Science 12:235-239.

Thseng, F.S. & Hosokawa. 1972. Significance of growth habit in soybean breeding indeterminate varietal differences in characteristic of growth habit. Japan Journal Breed. 22:261-268.

Woodworth, C.M. 1932. Genetics and Breeding in improvement of soybean. University of Illinois Agr. Exp. Sta. Bull. 384:297-404.

Gai Junyi
Liu Shunhu
Jin Junpei
Cui Zhanglin
Ma Yuhua

245

Gene frequency of SBTi-A_2 allele in soybean (G.max) seed storage protein in China

It has been testified by several authors (Singh, Hymowitz, Wilson, Hadely, Orf, Kaizuma, et. al.) that the Kunitz trypsin inhibitor (SBTi-A_2) is present in soybean seed in four allelic forms (Ti^a, Ti^b, Ti^c and ti) in which Ti^a, Ti^b and Ti^c are inherited codominately and the ti (null) is inherited as a recessive allele. The special genotype with no trypsin inhibitor (ti) was found.

Results:

Gene frequency of SBTi-A_2 allele of cultivated soybean in China.

There are more than 17,000 native cultivars of soybeans in China. The 9,180 genotypes from northeast, north, west, east and south of China were analyzed for SBTi-A_2 allele by author using PAGE. Three thousand two hundred and two genotypes from Shanxi, Hubei, Xizhang and Shanghai were analyzed by Liu Xingyuan (1992) using the same method, and the 3,962 genotypes from the eastern part of China, including five provinces and one city, were analyzed by Lin Guoqing (in press). There are 16,296 genotypes belonging to Ti^a type (99.71%), 41 genotypes belonging to Ti^b type (0.25%), 2 genotypes belonging to Ti^c type (0.01%) and 4 genotypes belonging to Ti^{a+b} type (0.024%) in Chinese soybean germplasm. One genotype with new electrophoretic form was found (Zhao shuwen et. al., 1992). There are no ti genotypes found in Chinese soybean germplasm. The variation of SBTi-A_2 alleles in Gansu, Heilongjiang, Jiangsu and Anhui was greater than that in other provinces, and some new electrophoretic variants were also found in these provinces.

Discussion: 1. The Rf value of electrophoretic band of one genotype from Gansu province is different from that of bands Ti^a, Ti^b, Ti^c (Zhao et. al. 1992). There are four genotypes from Jiangsu and Anhui provinces having both Ti^a and Ti^b (Ti^{a+b}) bands (Lin Quoqing, in press). It seems that there may be new allele of SBTi-A_2 existing in Chinese soybean germplasm. The biochemical and genetic analyses were needed for these special genotypes.

Table 1. Gene frequency of SBTi-A2 allele of soybean germplasm from different provinces of China

Provinces	No. of Sample	Ti^a		Ti^b		Ti^c		Ti^{a+b}		New type	
		No.	%	No	%	No	%	No	%	No	%
Liaoning*	744	743	99.87			1	0.13				
Jilin*	814	812	99.75	2	0.25						
Heilongjiang*	719	712	99.03	6	0.83	1	0.14				
Hebei*	749	749	100.0								
Neimeng*	190	190	100.0								
Shannxi*	950	950	100.0								
Gansu*	250	229	91.6	20	8.00					1	0.40
Ningxia*	99	99	100.0.								
Xinjiang*	20	20	100.0								
Shichuan*	870	870	100.0								
Yunnan*	300	299	99.7	1	0.30						
Guizhou*	1318	1318	100.0								
Guangdong*	231	231	100.0								
Guangxi*	453	453	100.0								
Zhejiang*	610	610	100.0								
Hunan*	337	337	100.0								
Henan*	526	525	99.8	1	0.20						
Shanxi**	1927	1927	100.0								
Hubei**	1223	1223	100.0								
Xizhang**	11	11	100.0								
Shanghai**	41	41	100.0								
Jiangsu***	1298	1292	99.5	4	0.30			2	0.30		
Anhui***	676	672	99.4	2	0.30			2	0.30		
Shandong***	769	765	99.5	4	0.50						
Jiangxi***	329	329	100.0								
Fujinan***	240	239	99.6	1	0.40						
Beijing***	650	650	100.0								
Total	16344	16329	99.71	41	0.25	2	0.01	4	0.02	1	0.01

Analysts: *zhao shuwen **Liu xingyuan ***Lin Guoqing

2. The 41 genotypes with Ti^b are distributed in many regions of China. This seems that the formation of this electrophoretic form is not related to the latitude and longitude. These genotypes distributed mainly in the province with large ecological and geographical diversity, such as Gansu, Heilongjiang and Yunnan province.

3. Comparison of Ti^b frequence of G.soja and G.max. from the same province.

Table 2

Provinces	G. soja No. of samples	Ti^b		G. max No. of samples	Ti^b	
		No.	%		No.	%
Gansu	90	0	0	250	20	8.0
Shannxi	400	37	9.25	950	0	0
Shanxi	422	28	6.64	1927	0	0
Shandong	70	6	8.57	769	4	0.52
Heilong-jiang	739	12	1.62	719	6	0.83
Ningxia	24	0	0	99	0	0
Shichuan	35	0	0	870	0	0

(Liu, Lin, Zhao, et al. in press)

We can see from Table 2 that there are no regular relationships of Ti^b frequency between G.soja and G.max. New regular results may be found if the semi-wild soybean from the same province were analyzed.

References

Hymowitz, T. and H. H. Hadley. 1972. Crop Sci. 12:197-198
Hymowitz, T. and N. Kaizuma. 1978. Soybean Genet. Newsl. 5:19-24.
Liu Xingyuan. 1992. Soybean Sci. China 11 (2):134-137.
Singh, L. et. al. 1969. Crop Sci. 9:489-491.
Zhao Shuwen et. al. 1991. Soybean Genet. Newsl. 18:111-112.
Zhao Shuwen et. al. 1992. Soybean Genet. Newsl. 19:22-24.

Zhao Shuwen (JiLin)
Liu Xingyuan (Hubei)
Lin Guoqing (Nanging)

Heilongjiang Academy of Agricultural Sciences
Soybean Research Institute
Harbin, Heilonjiang Province
The People's Republic of China

245

Waterlogging damage of soybean and variety resistance

Soybean need a large amount of water, and there is positive correlation between soybean yield and moisture of soil. However, when moisture is too high or the soil is soaked by water, the roots of soybean can not grow well and their function is hindered causing waterlogging damage.

There are some more studies and reports on dry damage and drought-resistance of soybean and methods to measure them to add to former studies on water physiology and water stress. Here we'd like to talk more about waterlogging damage and waterlogging-resistance of soybean varieties as well as methods to measure waterlogging-resistance in order to give some reference for developing waterlogging-resistant varieties and managing soybean in the low-lying areas susceptible to waterlogging.

Materials and methods: The most common soybean varieties cultured in Heilongjiang Province recently were cultured in pots and soaked in different growing periods for two years. There were four plants in each pot and three replications in each year. In the first year, soaking began from V1, R1 and R3, respectively. The plants were soaked for 20 days or until plant maturity. In the second year it was only from V1 until maturity.

The yield and agronomic characters were measured in maturity. Data was computed by MSTAT program in IBM.

Results and discussion: When soybean was soaked in growing period, its yield would be reduced (Table 1). The damage of soaking can be measured by damage degree (K):

$$K = (C-T)/C*100\%$$

K = Damage degree by percentage

C = Value of control of plants

T = Value of treatment plants

After soaking, the damage degree of plant yield was 50.20% - 67.99%. It meant the yield of soaking plant was 32.01% - 49.80% of yield of normal plant (ie - yield was reduced by one-half to two-thirds). The reason for the reduced yield was that the plant could not develop well. They lost flowers and many pods dropped. The damage degree of yield characters and the characters related to yield, such as plant weight, pods of plant, number of plant, stem weight, and so on was around 60%. Seed weight was the only exception among them. The weight of 100 seeds for soaked plant was more than for CK because there were less pods and seeds on soaked plants than on normal plants and photosynthetic produce made capacity of pool larger.

Table 1. Damage Degree of Soaked Soybean

Damage degree	Plant height	No. of Nodes	No. of branches	Plant weight	Pods/ Plant	Pods with 3-4 seeds	Seeds/ Plant	Yield/ Plant	Stem Weight	Weight/ 100 seeds
Average	12.79	16.29	74.71	59.08	64.38	61.16	62.29	60.37	56.41	-4.43
Lowest	-0.43	11.52	39.15	44.35	58.24	51.29	55.85	50.20	42.00	-13.72
Highest	20.60	21.14	100.00	63.00	68.46	66.35	68.92	67.99	62.08	1.63
CV %	24.26	29.57	15.10	12.35	10.36	28.04	13.23	19.24	10.57	133.15

Among morphological characters, number of branches was damaged the most seriously. Plant height and number of nodes were damaged by soaking, but damage degree was only 12.79% - 16.26%.

Damage degree of major agronomic characters was different among different varieties soaked. It suggested that there was difference in waterlogging resistance of soybean varieties. Among 11 characters studied, there were four characters, nodes of stem, number of branches, number of pods with 3-4 seeds, and plant yield, which were noticeable significant differences among varieties. Three characters, pods of plant, plant weight and seeds of plant, were significant differences among varieties. Other characters did not have significant differences among varieties. Among eight varieties there was only one, Baojiao 83-5029, in which yield of plant was the highest. In soaking condition, this variety gave higher plant, more nodes and pods, more pods with 3-4 seeds for every plant. This means that we can get more seeds and a higher yield from it (i.e. Baojiao 83-5029 is a waterlogging-resistant type). Against it, Nenfeng 12 and Ke 8118 will be damaged by waterlogging easily (Table 2).

Table 2. Performance of Major Agronomic Characters of Eight Soybean Varieties

Variety Name [a]	Plant Height	No. of Nodes	No. of Branch	Plant Weight	Pods/ Plant	Pods with 3-4 seeds	Seeds /plant	Yield/ plant	Stem Weight	Weight /100 seeds	Ratio of stem thick [b]
NF12	52.07	12.24	0.90	8.77	11.16	6.34	27.33	3.26	5.59	12.00	1.32
Ke8118	65.39	11.49	0.21	8.10	8.07	3.31	18.03	2.57	5.56	15.11	1.30
HF22	57.60	11.59	1.64	10.10	11.44	3.76	23.90	3.54	6.56	15.67	1.31
HF25	60.29	13.74	0.13	10.56	13.07	4.79	27.51	4.16	6.37	15.37	1.32
HN16	67.91	14.21	1.67	10.74	13.69	5.96	30.14	4.14	6.64	13.86	1.39
BJ 835029	77.53	14.84	0.87	14.10	13.14	8.40	33.76	5.87	8.06	16.77	1.43
DN 82-833	67.44	14.44	1.99	12.37	16.36	5.31	32.84	4.77	7.60	13.10	1.35
HN26	70.51	13.66	1.06	11.87	13.96	5.23	29.73	4.14	7.56	13.63	1.35
Avg	64.84	13.28	1.06	10.83	12.61	5.39	27.91	4.06	6.74	14.44	1.35
F-Value	1.82	4.45	4.38	2.30	2.75	4.46	2.94	4.37	0.66	1.09	0.31

Notice: [a] NF = Nenfeng, HF = Hefeng, HN = Heinong, DN = Dongnong, BJ 83-5029 = Baojiao 83-5029 (Bafeng 2)

[b] Ratio of stem thickness meant the ratio between the stem thickness, upper water face and the stem thickness under water face.

There was no significant difference between different soaking days for plant height, number of nodes and branches. For these characters, damage degree of the treatment soaked from V3 for 20 days was no different than that soaked from V3 until maturity period. The damage degree for these characters was significant difference among the treatments soaked from different growing periods. For the treatment soaked from V3, plants were the shortest and nodes and branches were the least even through soaking for 20 days or until maturity. The value of above-mentioned characters of treatments soaked from R1 was higher than that from V3, but lower than that from R3. It suggested that the earlier plant was soaked, the more serious growing and developing of plant was damaged.

The damage degree of the treatment, soaked until maturity, was more serious than that by 20 days, for number of pods, number of seeds, yield of plant and 100 seed weight. Among them were two characters, seed number and seed weight per plant, which were not significant differences among those treatments from V1, R1 and R3. It meant that yield would be reduced seriously when soybean was soaked in any period

from the last stage of nutritional growing to the middle stage of reproductional growing. For plant weight and stem weight, the earlier soybean was soaked, the more characters were damaged. For number of pods of plant, the most serious period to be damaged was soaking from R1. For 100 seed weight, it was from R3.

Table 3. Significance of Differences among Soaking Treatments for Major Agronomic Characters of Soybeans

F value	Plant height	No. of nodes	No. of branch	Plant weight	Pods/ plant	Pods w/ 3-4 seed	Seeds /plant	Yield/ plant	Stem weight	Weight / 100 seeds	Ratio of stem thick
Days	1.8	9.1	0.0	8.7**	7.7**	9.5**	11.1**	22.4**	1.0	10.9**	28.2**
Period	55.9**	15.3**	12.9**	8.10	8.0**	2.1	0.93	1.3	85.9**	11.1**	25.2**
DxP[a]	1.1	0.8	0.1	10.10	4.4*	5.1**	5.6**	6.9**	1.0	1.9	0.1

Notice: [a] DxP means Days x Period
* and ** mean significant level at 0.05 and 0.01

Above results showed that waterlogging begun from V3 would damage growing and developing of soybean seriously. If waterlogging time was more than 20 days, soybean production would not be made up. When waterlogging occurred in R1 period, the pods set on soybean plant were reduced. But, if waterlogging time is not too long, some higher yield will be produced. In these cases, seed can develop fully due to the ability of its self-regulation and the increase of seed weight can make up a part of loss caused by pod reducing. For the soybean soaked at V1 for a short time, the yield reduced would not be too important. After soaking plant was removed, there was a longer time to recover. Each plant could set more pods, more seeds would be set in each pod and seeds would develop enough. When the soybeans were damaged by waterlogging in R3 for a short time, 100 seed weight and yield were reduced more seriously even though some pods would set on the plant. In the case that plants were soaked until maturity time, there was not a chance to recover normal growing and make up a loss for plants. So the earlier the waterlogging, the more serious the plant damage. (Table 4)

Table 4. Performance of major agronomic characters of soybean soaked in different treatments

Treat-ment[a]	Plant Height	No. of Nodes	No. of Branch	Pods/ Plant	Pods w/ 3-4 seeds	Plant weight	Yield/ plant	Seeds /plant	Stem weight	Weight /100 seeds	Ratio of stem thick
				Soaking Days							
20 days	63.8	13.3	0.9	12.9	6.0	11.1	4.8	29.4	6.2	16.5	1.3
matur-ity	60.5	12.6	0.9	10.5	4.2	9.1	3.2	22.9	5.9	13.6	1.5
				Soaking Period							
V3	47.8	11.9	0.3	10.8	5.4	7.7	4.0	25.3	3.8	14.9	1.5
R1	50.1	12.3	0.7	10.1	5.7	9.7	4.4	25.1	5.3	17.6	1.3
R3	79.5	14.6	1.7	14.1	4.3	12.9	3.7	28.0	9.1	12.6	
				Days X Periods							
20-V3	51.9	12.6	0.3	13.7	7.3	9.8	5.6	32.5	4.2	17.3	1.4
20-R1	58.7	12.8	0.8	11.3	6.8	10.3	5.0	28.6	5.1	18.0	1.2
20-R3	80.8	14.6	1.6	13.8	4.0	13.1	3.9	27.2	9.2	14.1	
M-V3	43.8	11.3	0.4	8.0	3.5	5.6	2.3	18.2	3.3	12.4	1.6
M-R1	59.4	11.8	0.7	9.0	4.6	9.1	3.7	21.6	5.4	17.2	1.4
M-R3	78.3	14.6	1.8	14.5	4.6	12.7	3.6	28.9	8.9	11.2	

Notice: [a]M means the treatment soaked until maturity

The correlation for damage degree of major agronomic characters as well as among the morphological characters of plant height, number of nodes and number of branch of soybeans soaked was significant. It showed the same reaction to soak for these two characters that damage degree of plant height related to that of node number significantly. After soaking, plants became short. It was in relation to the reduction of nodes to a considerable degree. Damage degree of branch related significantly negatively to that of plant height and node number. It meant that reducing branch would probably be an ability of self-regulation. In the bad condition with waterlogging, none or only a few branch could be formed to complete whole growing cycle. It was proved by the facts that damage degree of branch was the highest in ten characters, and the damage degree of plant height and number of nodes. Most correlations among production characters were significantly positive. There were significantly positive correlation between damage degree of plant yield and pods of plant, number of pods with three to four seeds, seed number of plant. So it can be considered as the main

reason that seed and pod number was damaged by waterlogging. In normal condition, 100-seed weight, a character affected on yield more importantly, would not be damaged seriously by waterlogging. So that there was no significant correlation between the damage degrees of 100-seed weight and plant yield (Table 5).

Table 5. Correlation among damage degree of major agronomic characters of soybeans soaked

	No. of Nodes	No. of Branch	Plant Weight	Pod/Plnt	Pods with 3-4 Seeds	Seeds/ Plant	Yield/ Plant	Stem Weight	Weight/ 100 Seeds
Plant Height	.718**	-.508*	.219	-.105	.051	-.116	-.180	.499*	.054
No. of Nodes		-.485	.550*	.338	.407	.243	.199	.584**	.195
No. of Branch			-.241	.171	-.001	.268	.233	-.385	-.366
Plant Weight				.794**	.600**	.612**	.712**	.850**	.312
Pods/ Plant					.629**	.729**	.936**	.483**	.088
Pods with 3-4 Seed						.779**	.568*	.284	-.259
Seeds/ Plant							.714**	.213	-.161
Yield/ Plant								.390	.104
Stem Weight									.367

There was not significant correlation between morphological and yield characters. After soaking, plant became short with less nodes and branches. It was a self-regulation reaction to a bad condition. This ability was different among varieties. It was not found in our research that damage degree of yield related to these morphological characters noticeably, so we could not forecast the degree of damage of yield using that of plant height, number of nodes or branches in general means.

After soaking, a most special reaction of soybean was the part of stem soaked in water became noticeably thicker. We called the ratio between the part of stem soaked in water and the part of stem above water as ratio of stem thickness (RST). When the soybean plants were soaked from different growing periods on different days, the RST of treatments was significantl difference (Table 3). High value of RST meant the part of

stem under face of the water thickened more seriously and the plant was severely damaged. It can be known from Table 4 that there was a tendency to negative correlation between RST and yield of plant in the treatments soaked from different growing periods or for different days. So as far as waterlogging damage of soybeans, high RST value meant more serious waterlogging. In Table 2 there was a positive correlation between RST and yield of different varieties ($r=0.854^{**}$). So that RST can be used to measure the difference of waterlogging-resistance of soybean varieties. The varieties with high RST value will be the good one in waterlogging-resistance.

Conclusion: Waterlogging will reduce soybean yield. When the soybeans are soaked for a longer time, the yield will be reduced by one-half to two-thirds.

There is a noticeable difference of waterlogging-resistance among varieties. Waterlogging-resistant variety, Baojiao 83-5029, shows higher plant, more nodes, pods and seeds, and higher yield in the condition of waterlogging. The part of stem soaked in water of waterlogging-resistant variety becomes noticeably thick, the value of ratio of it and the part up water is bigger than others. The correlation between RST and yield of variety is the most significant. It can be a target to measure the resistance of waterlogging.

When the soybean is damaged by soaking, there are some reaction in morphological characters is almost same as that from V3 until maturity. For yield characters, damaged degree for long time soaking is more serious than that for 20 days-soaking. After V3 period, damage degree of morphological characters of soybean soaked for 20 days is same as that for a long time.

In the condition that soaking days are not more than 20 days, when the soaking is begun from V3, the soybean yield is reduced seriously. But the period to reduce yield the most seriously for soaking soybean is R3. In R3 stage, waterlogging reduces yield as reducing seed number of pod and 100 seed weight. Waterlogging of R1 stage reduces pod number seriously, but yield reduction is some less due to the recouperative action of seeds. So that, managing damaged soybean as earlier as possible after waterlogging can reduce the loss of yield. When soybeans are soaked in the period from V3 to R3 for a long time, yield is reduced more seriously. The earlier waterlogging occurs, the more soybean yield will be reduced.

References

Kawase, M. 1981. Anatomical and morphological adaptation of plants to waterlogging. Hor. Sci. 16:8-12.

Song Yingshu et al. 1990. Studies on influence to soybean development and tolerance to waterlogging in low water sok. Heilongjiang Agr. Sci. 2:16-20.

Xu Yonghua, He Zhihong et al. 1991. Waterlogging resistance and peroxidase activity in soybean. Soybean Genetics Newsletter. 14:78-81.

Xu Yonghua
He Zhihong
Song Yingshu
Du Zhiqin
Yin Dalong

Croatia

241

Reconstruction and development of the Osijek Agricultural Institute after the war in Croatia

The Osijek Agricultural Institute (Eastern Croatia) is an independent scientific and research organization with a long and rich tradition, having existed for 115 years. It deals with scientific and professional activities in the areas of the cultivation of land such as plant production and animal husbandry. It is well known both in the domestic and foreign scientific and professional fields for its scientific results (more than 170 of its own varieties and hybrids, as well as new technologies in plant production and animal husbandry).

During the 1991 war in Croatia, the Institute was completely destroyed - all buildings, laboratories, facilities for seed processing, scientific equipment and agricultural machines. The test fields, including other fields (approximately 800 ha), are inaccessible since this is the first front line of the Osijek's defense.

According to the rough estimates taken at the beginning of 1992, the total damage of the Institute amounts to approximately 26 million of DM (this does not include the premises under temporary occupation).

On the account of the willingness of the scientists, the Institute has begun the reconstruction and resumption of its activities. The main conception of the reconstruction and development involves the following components: the resumption of the scientific and research activities in the area of plant production as well as the development of some new ones; the reconstruction and building of infrastructural objects of the material basis and providing for the test fields.

The modern and reconstructed Institute would work on the following activities:

-plant breeding and seed production (creation of new varieties and hybrids, improvement of the genotypes of wheat, barley, maize, alfalfa, soybean, sunflower, and seed production)

-agroecological investigations (plant and soil analyses, recommendations for the tillage and fertilization, soil improvement, irrigation, integral technique applied in plant production)

-the organization of the production, seed processing and marketing (the production of high categories of the seed originating from the Institute's varieties and hybrids, production and processing of the commercial seed, sale and marketing of the seed)

-the international relations and relationships (establishment and keeping of the relations with the scientific firms in Europe and all over the world, with the competent European and world institutes, universities, academies, various firms dealing with industry and seed production)

-the application of knowledge and skills to plant production (the application of our own results of the investigations; the cooperation with agricultural plants and farmers, training of the producers)

-the introduction of crops (agricultural, vegetable and fruit crops for further selection; the introduction of foreign varieties, hybrids, seedlings and seed for higher yields)

-the information system (the receipt and distribution of the scientific and profession information as a result of the investigations at the Institute; the receipt of the accomplishments from abroad from the computers and the Institute's library)

For the realization of the above-mentioned activities at the Institute, which will be reconstructed, the following scientific and intellectual team is necessary: 20 doctors of sciences, 20 magistrates of science, 20 assistants, 40 technicians, laboratory technicians and additional assistants, which is altogether 150 persons employed.

Since the complete infrastructure of the Institute is destroyed, either a sanation or rebuilding of new objects occupying an area approximately 11.000 square meters is necessary (cabinets, laboratories, workshops, storages, seed processing facilities, etc).

For the normalization of work, new scientific equipment is necessary for investigation of the physical and chemical soil and plant properties, the grain quality of wheat, maize and barley, the quality of oil crops and other crops included in the scientific program of the Institute. Also, the acquisition of the agricultural machines (22 tractors of various capacity, 4 combines for grain harvesting, 12 tractor trailers, various machines, ploughs, cultivators, etc) as well as of the special mechanization for sowing and

harvesting of the selection material is necessary.

In case that the Institute's fields are still inaccessible, we should provide for the adequate areas for trials (at a new location) occupying approximately 1000 ha.

The Scientific Council of the Osijek
Agricultural Institute

NATIONAL RESEARCH CENTRE FOR SOYBEAN
Khandwa Road
Indore, India

India

245

Stability of soybean varieties across diverse locations in India

In India, soybean cultivation is rapidly spreading to occupy third place in national production of oilseeds and edible oil. Most of the studies on stability of soybean varieties are, however, confined to a single location or few locations within specific region like Madhya Pradesh, Gujarat and Maharashtra (Rohewal 1970, Gopani et al. 1972, Lal et al. 1974, Bhatnagar and Tiwari 1989, Taware et al. 1991). In the present study, stability of improved soybean varieties, released or in pipe line, were studied across 9 geographically diverse locations of India.

Materials and Methods: The trial was conducted at each location in a randomized block design with three replications during rainy season of 1991. Individual plot size was 1.8 m x 5 m with a spacing of 45 cm between rows and 5 cm between plants. Stability parameters were computed following Eberhart and Russell (1966). The 18 varieties or strains included in the study are Bragg, Durga, JS 75-46, JS 80-21, JS 335, MACS 13, MACS 58, MACS 124, Monetta, PK 262, PK 416, PK 472, PK 564, Pb 1, Pusa 16, Pusa 20, NRC 1 and NRC 2. The nine locations and their respective environmental indices are Raipur (+3.74) in Madhya Pradesh, Kolhapur (+2.82) and Sangli (+1.60) in Maharashtra, Bhawanipatna (+1.21) and Bhubaneswar (-4.04) in Orissa, Ugarkhurd (-0.83) in Karnataka, Coimbatore (-0.87) in Tamil Nadu, Pratapgarh (-1.59) in Rajasthan and Jorhat (-2.05) in Assam.

Results and Discussion: The analysis of variance for yield has revealed significant differences among the varieties as well as among the locations. Significance of the variety x environment interaction component indicates differential genotypic adaptability across the locations. Both the linear and nonlinear components of the interaction were highly significant but the linear component was predominant over the pooled deviation component. The three parameters of stability viz. mean (X), linear regression coefficient (b - measure of response of a particular genotype) and deviation from linear regression (S^2d- parameter of stability of response) are presented in Table 1. Among the 18

varieties, 10 varieties showed mean yields above population mean and 9 varieties recorded nonsignificant S^2d values. The mean performance of a genotype along with other two parameters (b and S^2d) simultaneously considered to represent a measure of adaptability of the genotype. The varieties PK 416, PK 472, Pb 1, PK 262, Durga, PK 564, Monetta, NRC 1, and NRC 2 showed nonsignificant S^2d values to indicate that their yield responses were predictable in nature. Most of the high yielding varieties with grain yield above mean namely MACS 58, JS 335, JS 80-21, MACS 13, Durga, MACS 124 and NRC 1 were highly responsive (b values greater than unity). However, only in varieties Durga and NRC 1 the responses were predictable as they showed nonsignificant S^2d values; these two varieties are suitable for cultivation in favorable environments. The varieties PK 472, PK 416, PK 262, Pb 1 and NRC 2 showed nonsignificant b values. Except the cultivars PK 472 and Pb 1 these varieties were found to be poor yielders. Variety PK 472 and Pb 1 showed high and average yield respectively but both were less responsive to environmental changes. The variety JS 75-46 showed b value near unity and was found to be poor yielder with unpredictable performance. In the present study, none of the varieties fulfill the criterion of wide adaption viz. high mean, b near unity and nonsignificant S^2d values. The findings are in conformity with the earlier reports that soybean genotypes are by and large location specific (Cooper 1976). Among the existing varieties, PK 472 and Pb 1 have relatively wide adaption.

Acknowledgement: The contributions of various scientists by way of conducting the trials at various locations and recording observations are thankfully acknowledged.

Table 1. Yield (q/ha) and stability of performance of eighteen soybean varieties at nine locations in India.

Varieties	Raipur	Kolhapur	Sangli	Bhawani patna	Ugar-khurd	Coimb-atore	Pratap garh	Jorhat	Bhuba-neswar	Mean	b	S^2d
Bragg	15.25	21.22	17.00	14.35	16.77	12.29	12.45	19.71	10.11	15.46	0.71	7.26*
PK 416	14.51	14.47	13.18	10.92	12.66	11.71	12.69	10.74	14.31	12.80	0.76	-1.76
PK 472	20.37	15.64	15.07	14.91	20.07	17.23	13.63	10.54	13.44	15.66	0.66	4.34
Pb 1	13.27	17.05	17.29	18.24	11.74	14.96	15.60	14.35	8.66	14.57	0.71	2.83
MACS 58	18.77	25.55	23.22	16.95	12.37	14.20	13.47	6.84	7.85	15.47	2.19**	7.62*
PK 262	18.02	11.53	13.99	12.22	14.28	12.80	12.06	7.56	11.67	12.68	0.64	1.89
JS 335	24.14	23.44	15.29	22.96	15.63	16.24	17.18	15.67	6.70	17.47	1.88**	4.55*
JS 80-21	22.22	30.16	25.44	23.05	10.89	15.27	7.57	14.16	16.67	18.38	2.11**	25.22*
MACS 13	18.52	35.84	27.47	24.17	18.22	15.68	11.03	14.35	12.06	19.70	2.36**	30.35*
Durga	22.37	25.18	20.66	18.06	14.44	14.57	12.14	14.88	9.55	16.87	1.92**	-1.02
Pusa 16	14.51	2.09	5.55	12.41	10.96	11.15	8.90	9.91	6.22	9.08	0.11	12.86*
Pusa 20	15.62	4.70	3.03	6.85	11.78	11.23	7.64	4.71	11.81	8.60	-0.08	16.24*
PK 564	7.47	2.58	5.33	7.04	12.18	8.02	6.46	12.69	10.33	8.01	-0.85	2.96
Monetta	9.38	2.78	4.00	8.43	9.14	5.49	10.72	7.76	6.74	7.16	-0.26	3.48
JS 75-46	19.02	10.98	19.70	13.89	10.00	13.62	14.89	14.67	6.67	13.71	0.98*	8.63*
MACS 124	22.23	23.12	22.21	23.33	7.85	16.96	16.16	12.55	8.15	16.95	2.11**	8.73*
NRC 1	26.36	24.69	23.21	16.67	14.30	15.27	17.73	16.42	8.26	18.10	2.09**	2.05
NRC 2	19.45	14.02	11.36	11.48	15.85	11.87	15.13	9.74	12.30	13.47	0.54	4.00
Mean	17.86	16.94	15.72	15.33	13.29	13.25	12.53	12.07	10.08	14.12		

**P = 0.01, *P = 0.05

References

Bhatnagar, P.S. and S. P. Tiwari. 1989. Phenotypic stability analysis of yield in soybean (Glycine max (L.) Merill). Biovigyanam 15(2):90-93.

Cooper, R. L. 1976. Modifying morphological and physiological characteristics to maximize yields. (in) World Soybean Research. Hill, L. D. (Ed.). Interstate Printers and Publishers, Inc. pp 230-236.

Eberhart, S. A. and W. A. Russell. 1966. Stability parameters for comparing varieties. Crop Sci. 6:36-40.

Copani, D. D., M. M. Kabaria, and S. N. Joshi. 1972. Stability parameters for comparing varieties of soybean (Glycine max (L.) Merr). Ind. J. of Agric. Sci. 42(5):400-404.

Lal, M. S., S. K. Mehta, and S. C. Pandya. 1974. Stability of performance of soybean varieties in Madhya Pradesh. Ind. J. Agric. Sci. 44:217-220.

Rohewal, S. S. 1970. Stability of some superior soybean varieties. Ind. J. Genet. Plant Breeding 30:650-653.

Taware, S. P., G. B. Halvankar, V. M. Raut, and V. P. Patil. 1991. Analysis of phenotypic stability in soybean (Glycine max). Ind. J. Agric. Sci. 61(12):933-936.

P. S. Bhatnagar

P. G. Karmakar

NATIONAL RESEARCH CENTRE FOR SOYBEAN
Khandwa Road
Indore, India

245

Morphological and agronomic characteristics of soybean varieties of India

Forty-four released/identified varieties of soybean suitable for cultivation in five agroclimatic zones of India are being maintained at National Research Center for Soybean at Indore. In this paper, the descriptive data on ten qualitative characters and agronomic data on six economically important quantitative characters of these varieties have been summarized.

Descriptive data: The descriptive data on ten important qualitative characters of each variety is presented in Table 1. The underlined letters, as described below, were used as descriptive code following Coble et al. (1991). These codes were arranged in the sequence of 1 23456 789 10.

The details of the characters are given below:

1.	Stem termination:	INdeterminate, Semi-determinate, Determinate
2.	Flower color:	Purple, White
3.	Pubescence color:	Tawny, Gray
4.	Pubescence form:	Appressed, Erect, Semi-appressed
5.	Pubescence density:	Dense, Glabrous, Normal, Semi-dense, Sparse
6.	Pod color:	Black, Brown
7.	Seed coat luster:	Dull, Shiny, Intermediate
8.	Seed coat colour:	Yellow, Black
9.	Hilum colour:	Gray, Black, Brown with prefix Light, Dark, Reddish
10.	Other characters:	Narrow leaf

Agronomic data: These 44 varieties were grown at Indore (latitude 22° 41' N, longitude 75° 52' E, altitude 540 m, average rainfall 800 mm) during rainy season (June - October) for two years in 1991 and 1992 and data were recorded on days taken from sowing to 50% flowering and maturity, vine length (cm) at harvest, 100 seed weight (g),

harvest index (%) and seed yield (q/ha). The performance of the varieties was showing similar trend in both the years, hence the average data of the two years is given in Table 2. The early maturing variety JS 71-05 was earliest flowering (34.4 days), dwarf (43.0 cm) and had very bold seeds (16.5 g per 100 seed weight) while variety Monetta matured earliest (91 days). T 49, one of the old variety, was late in flowering (55.4 days) and maturity (122.9 days) and also showed very low harvest index (14.79 %) as well as yield (10 q/ha). Co 1, another early released variety gave lowest seed yield (8.7 q/ha) as well as test weight of seed (6 g), low harvest index (16.7 %) and long vine growth (125.3 cm) at harvest. The top yielding varieties, namely PK 471, PK 564, Pusa 24 and JS 335 gave high harvest index (33.8 to 36.6 %) and were relatively recent releases or identification. Some of the top ranking varieties for yield were initially released for zones other than central zone of India.

References

Coble, J., T. Claudia, Gerald L. Sprau, Randall L. Nelson, James H. Orf, Donna I. Thomas and James F. Cavins (1991). Evaluation of the USDA Soybean Germplasm Collection: Maturity Groups 000 to IV (PI 490.765 to PI 507.573. US Department of Agriculture Technical Bulletin No. TB-1802, 61 p.

P. G. Karmakar
P. S. Bhatnagar
Vipin Kumar

Table 1. Descriptive data on 44 soybean varieties of India

Variety	Descriptive code		Variety	Descriptive code	
Alankar	D WTEDnBr	YSB1	MACS 58	S PTSaDnB1	YSLBr
Ankur	D WTENBr	YDBr	MACS 124	S PTADnBr	YSBr
Birsa Soybean 1	D WTESdnBr	B1IB1	Monetta	D PTADnB1	YSG
Bragg	D WTENBr	YSB1	Punjab 1	S PTADnBr	YSBr
Co 1	N PTSaDnBr	YIDBr	PK 262	D WGEDnB1	YSBr
Durga	S WTEDnBr	YDB1	PK 308	D WGEDnG	YSRBrN1
Gaurav	D PTEDnBr	YDB1	PK 327	D PGENG	YDBr
Gujarat Soybean 1	N PTSaNBr	YIBr	PK 416	D WTEDnBr	YSB1
Gujarat Soybean 2	N PTEDnB1	YIBr	PK 471	D WTSaDnBr	YSB1
Hardee	D WGEDnG	YDRBr	PK 472	D WGEDnG	YSRBr
Improved Pellcan	N PTENBr	YSDBr	PK 564	D WTESdnBr	YSBr
JS 2	D PTADnBr	YSLBr	Pusa 16	S PTENBr	YIG/B1
JS 71-05	D PTASpBr	YSB1	Pusa 20	D WTENBr	YIB1
JS 75-46	S PGEDnB1	YSBr	Pusa 22	D PTEDnBr	YDG
JS 76-205	S PTENBr	B1SB1	Pusa 24	D WTENBr	YIB1
JS 80-21	D PTEDnBr	YIBr/B1	Pusa 37	D WTENBr	YIB1
JS 335	S PTASpBr	YSG	Pusa 40	D PTEDnBr	YSBr
Kalitur	D PTENB1	B1IB1	Shilajeet	D PTADnBr	YIBr
KHSb 2	S PTEDnB1	YDB1	Shivalik	D WGEDnG	YSBr
Lee	S PTEDnB1	YIB1	T 49	N PGENB1	YSLG
MACS 13	D PTENB1	YIB1	VL Soya 1	D WTSaDnBr	B1IB1
MACS 57	S PTADnBr	YSBr	VL Soya 2	D PGESdnG	YDB1

Table 2. Agronomic characteristics of 44 soybean varieties of India (average data of two years trials conducted at Indore, Madhya Pradesh, latitute 22° 41' N, longitude 75° 52' E, altitude 540m)

Variety	Flowering (day)	Maturity (day)	Vine length (cm)	100 seed weight (g)	Harvest index (%)	Seed yield q/ha	Zone of adaptation
Alankar	47.0	105.7	77.9	12.2	29.1	18.2	II
Ankur	44.0	107.0	79.6	13.3	32.2	23.0	II
Birsa Soybean 1	44.4	107.4	68.0	13.2	25.8	15.3	BP
Bragg	37.9	101.9	72.4	14.1	27.1	19.6	I,II,III,IV
Co 1	50.0	103.5	125.3	6.0	16.7	8.7	TN
Durga	48.0	106.2	87.2	9.7	27.4	19.2	III
Gaurav	44.4	106.9	95.7	9.2	23.6	13.0	III
Gujarat Soybean 1	48.0	111.0	117.6	9.7	18.6	13.0	G
Gujarat Soybean 2	47.0	110.0	118.8	9.8	21.4	15.4	G
Hardee	43.0	114.5	84.7	15.1	27.8	20.1	IV
Improved Pelican	49.5	109.5	102.3	10.7	19.6	13.9	IV
JS 2	38.5	94.7	50.3	15.3	29.6	17.3	III
JS 71-05	34.4	99.0	43.0	16.5	31.8	18.6	MalP
JS 75-46	45.0	108.9	112.7	14.7	26.3	20.7	III
JS 76-205	-	102.0	-	9.1	-	17.8	MP
JS 80-21	46.0	106.4	88.5	9.7	22.8	13.6	III
JS 335	47.0	103.0	73.7	12.8	36.3	21.8	III
Kalitur	44.5	100.0	98.7	10.7	27.7	18.4	MP
KHSb 2	51.4	110.7	91.6	10.8	20.6	12.6	K
Lee	50.0	110.0	116.1	10.5	20.3	13.3	I
MACS 13	48.0	106.5	77.2	11.8	26.7	17.4	III
MACS 57	45.5	98.5	89.6	10.0	23.1	16.1	M
MACS 58	45.0	106.0	101.4	10.9	24.7	14.3	III
MACS 124	49.0	102.0	109.4	12.3	30.5	18.9	IV
Monetta	36.0	91.0	73.5	12.4	29.6	20.5	III,IV
Punjab 1	44.2	98.0	85.4	10.3	26.2	14.2	I,II,III
PK 262	45.5	107.0	70.3	13.1	36.2	25.7	I,II
PK 308	42.0	99.2	69.3	10.1	32.1	22.6	I,II
PK 327	42.0	93.0	70.0	9.9	32.1	24.5	I,II
PK 416	39.4	105.7	76.1	14.5	36.1	23.8	I,II
PK 471	45.2	99.0	71.1	13.5	35.9	26.8	IV
PK 472	42.7	101.7	65.4	12.3	31.4	21.7	III
PK 564	43.9	102.5	74.3	11.8	36.6	25.2	II
Pusa 16	43.0	97.8	79.7	11.1	30.8	19.4	I,II,V,MP
Pusa 20	45.5	103.9	69.6	11.6	29.8	15.2	I

Table 2. (Continued)

Variety	Flowering (day)	Maturity (day)	Vine length (cm)	100 seed weight (g)	Harvest index (%)	Seed yield q/ha	Zone of adapta-tion
Pusa 22	53.5	107.5	107.6	12.1	25.1	16.5	II,III,V
Pusa 24	46.0	104.7	76.7	13.7	33.8	22.2	I,II,V,MP
Pusa 37	44.0	108.4	74.7	12.3	27.9	17.7	II,III,IV
Pusa 40	42.0	97.0	43.3	17.5	34.2	16.8	IV
Shilajeet	43.0	94.2	56.5	16.2	29.7	17.5	I,II
Shivaiik	44.0	99.5	63.4	13.5	32.8	22.9	HP
T 49	55.4	122.9	105.1	7.2	14.8	10.0	III
VL Soya 1	43.0	99.5	60.4	16.5	34.4	19.3	UP
VL Soya 2	41.5	101.0	56.5	17.7	27.8	14.7	I
Mean	44.9	103.8	82.1	12.2	28.0	18.0	
	+0.6	+0.9	+3.1	+0.4	+1.0	+0.7	

I	North Hill Zone	BP	Bihar Plateau	K	Karnataka
II	North Plain Zone	TN	Tamil Nadu	M	Maharashtra
III	Central Zone	G	Gujarat	HP	Himachal Pradesh
IV	Southern Zone	MalP	Malwa Plateau	UP	Uttar Pradesh
V	North Eastern Zone	MP	Madhya Pradesh		

PUNJAB AGRICULTURAL UNIVERSITY
Department of Plant Breeding
Ludhiana, India 141004

245

Screening of soybean germplasm for high-protein and high oil lines

Soybean [Glycine max (L) Merrill] seed, which contains about 40 percent protein and 20 percent oil on a dry weight basis, provides approximately 60 percent of the world's supply of vegetable protein and 30 percent of the oil (Foreign Agricultural Service, 1985). Most of this seed is processed to separate the protein and oil fractions. The protein, which is of high quality, is utilized for manufacturing protein products and has other industrial applications. The oil is used for margarine, shortenings, and other fat and oil products. Keeping in view the importance of soybean, efforts were made to identify lines having either high protein and/or oil content.

Materials and methods: Sixty genetically diverse genotypes of soybean were evaluated in a randomized complete block design on two different sowing dates during the normal crop seasons (June to October) of 1988 and 1989. Four environments were thus created. Each entry replicated thrice was accommodated in a single row of 3 meter length. Protein content from dry seed was determined by Kjeldahl's method while total oil content was determined by nuclear magnetic resonance (NMR) following Alexander et al (1967).

Results and discussion: The analysis of variance pooled over the four environments indicated that the genotypes differed significantly over environments for protein as well as oil content. Genotype environment interactions were also significant for both these important quality components. High heritability values for protein (44.4%) and oil (50.8%) indicated that there was sufficient genetic variability available in the material and therefore, selection of genotypes with high oil/protein is possible.

The protein content of different genotypes ranged from 34.5 to 41.4 percent with overall mean of 37.0 percent. 'DS-8' with protein content of 41.4 percent, had the highest level of protein, closely followed by 'SL-96' (41.3 percent). 'JS 81-716' (39.9 percent) and 'Pb-1' (39.7 percent), were also desirable genotypes having high level of protein. Out of sixty genotypes, about thirty-one genotypes had protein content above or equivalent to

grand mean while the rest were below grand mean.

The oil content for different genotypes ranged from 19.1 to 22.4 percent with overall mean around 20.9 percent. The highest level of oil was recorded in 'DS-9' closely followed by 'PK 566', 'PK 568', 'PK 695'(22.3 percent), 'PK 564'(22.2 percent) and 'PK 416' (22.1 percent). PK 416 is a very popular variety of soybean recommended in Northern India. The lowest oil content was recorded by DS-8. This genotype had the highest level of protein. This is due to strong negative correlation between oil and protein content (Sharma, 1991). About thirty-three genotypes had oil content higher or equivalent grand mean.

This study clearly indicated that there are remote chances to identify a genotype with both high protein and high oil content. The genotypes with high protein were DS-8, SL-96, Pb-1, and JS 81-716 while high oil genotypes were DS-9, PK 566, PK 566, PK 695 and PK 416.

These genotypes can be used in crossing programs in order to improve oil or protein level of otherwise adaptable and high yielding soybean lines depending upon the objective or utility.

References

Alexander, D.E., S.L. Silvela, F.I. Collina and R.C. Rodgers. 1967. Analysis of oil content of maize by wide line NMR. J. Am. Oil Chem. Soc. 44:555-558.

Foreign Agricultural Service. 1985. Foreign Agricultural Circular Fol. 1-85. USDA, Washington, D.C.

Sharma, S.R. 1991. Genetic analysis for yield attributes and quality traits in soybean [Glycine max (L0 Merrill)] Ph.D. Unpublished thesis, PAU, Ludhiana.

P.S. Phul
S.R. Sharma

NATIONAL RESEARCH CENTER FOR SOYBEAN (ICAR)
Indore 452001 (M.P.)
India

245

Multiple regression analysis for seed yield in soybean

Correlation analyses have been carried out in soybean (Bernard and Weiss, 1973), however, comprehensive regression analyses are further warranted with the purpose of narrowing the choice of selection criteria for genetic improvement in yield (Bernard and Weiss, 1973). Present study utilizes multiple regression analysis towards this end.

A sample of 80 soybean genotypes was planted in duplicate sets at our research farm in 1990. The mean values for seven yield and yield contributing attributes were used for multiple regression analysis by least squares technique (Chatterjee and Price, 1977).

The simple correlation coefficients obtained for all possible combinations of the seven characters are presented in Table 1. Seed yield was found to be significantly correlated with all the characters except 100 seed weight. Multiple correlation coefficient ('R') for the six characters versus yield was found to be 0.978, which was highly significant. Estimated value of R^2 was 0.956 indicating that almost all the variability for seed yield is accounted for by the variables included in the study.

All possible 63 combinations (26^2 -1) were studied for obtaining the maximum multiple correlation coefficient and least deviations mean square in order to select a desirable subset of variables for prediction of seed yield. Some of the selected subsets, especially those giving the 'R' value of 0.97 and above, are presented in Table 2. Interestingly, certain subsets with as few as three variables gave almost the same value of 'R' as obtained by the original set of six variables. The subset comprising pods per plant (X_4), seeds per pod (X_5) and 100 seed weight (X_6) was identified as the most desirable for prediction of yield, giving a 'R' estimate of 0.978. Although some other subsets involving four or five variable were almost as good, the present subset containing the least number of variables was preferred for simplicity as suggested by Snedecor and Cochran (1967). Further analysis showed that contribution of X_4, X_5 and X_6 towards multiple R^2 was 95.31%, 4.53% and 0.16%, respectively, indicating the maximum contribution to the character pods per plant towards seed yield. Confirmatory path

analysis, carried out as per Dewey and Lu (1959), also showed the maximum direct effect of pods per plant towards yield (Table 3). Earlier studies have reported the pods per plant (over all seed number) to be more important than seed size (Herbert and Litchfield, 1985). The presently identified subset consisting of the three yield components appears to be quite potent in prediction of seed yield in soybean. The regression equation for the subset was found to be seed yield (Y) = -12.9674 + 0.2342 X_4 (number of pods per plant) + 3.2230 X_5 (number of seeds per pod) + 0.6239 X_6 (100 seed weight).

References

Bernard, R.K. and M.G. Weiss. 1973. In: Soybean improvement, production and uses. B.E. Caldwell (ed.) Amer. Soc. Agron. Madison, Wisconsin, Series No. 16. pp 117-154.

Chatterjee, S. and B. Price. 1977. Regression analysis by example. Wiley, New York.

Dewey, B.R. and K.H. Lu. 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. Agron. J. 51:515-518.

Herbert, S.T. and C.V. Litchfield. 1982. Partitioning soybean seed yield components. Crop Sci. 22:1074-1079.

Snedecor, G.W. and W.G. Cochran. 1967. Statisical Methods, Iowa State University Press, Ames, Iowa USA. pp 412-414.

Prabhakar
S.P. Tiwari

Table 1. Correlation coefficients ('R') for yield and yield attributing characters in soybean

Characters	X_2	X_3	X_4	X_5	X_6	X_7
X_1	0.792**	0.539**	0.675**	0.003	-0.004	0.638**
X_2	-	0.660**	0.870**	0.005	-0.107	0.811**
X_3			0.556**	-0.022	-0.218*	0.480**
X_4				0.815	-0.082	0.958**
X_5					-0.1231	0.250*
X_6						0.014

**Significant at 0.01P *Significant at 0.05P

X1 = Days to flowering X4 = Number of pods per plant X7 = Seed Yield

X2 = Days to maturity X5 = Number of seeds per pod

X3 = Plant height X6 = 100 seed weight

Table 2. Multiple correlation coefficients ('R') between seed yield (X_7) and contributing characters (X_1 to X_6) in soybean

Combination of characters/subset	'R'	Combinations of characters/subset	'R'
$X_1+X_2+X_3+X_4+X_5+X_6$	0.978	$X_3+X_4+X_5$	0.972
$X_2+X_3+X_4+X_5+X_6$	0.978	$X_2+X_4+X_5$	0.971
$X_1+X_3+X_4+X_5+X_6$	0.978	$X_1+X_4+X_5$	0.971
$X_1+X_2+X_4+X_5+X_6$	0.978	$X_3+X_4+X_6$	0.963
$X_1+X_2+X_3+X_4+X_5$	0.973	$X_2+X_4+X_6$	0.963
$X_1+X_2+X_3+X_4+X_6$	0.964	$X_1+X_4+X_6$	0.963
$X_1+X_2+X_3+X_5+X_6$	0.859	$X_2+X_5+X_6$	0.858
		$X_1+X_5+X_6$	0.686
$X_2+X_3+X_4+X_5$	0.978	X_4+X_5	0.971
$X_3+X_4+X_5+X_6$	0.978	X_4+X_6	0.962
$X_2+X_4+X_5+X_6$	0.978	X_2+X_6	0.817
$X_1+X_4+X_5+X_6$	0.978	X_1+X_6	0.638
$X_1+X_3+X_4+X_5$	0.972	X_3+X_6	0.495
$X_1+X_2+X_5+X_6$	0.858	X_5+X_6	0.254
$\underline{X_4+X_5+X_6}$	$\underline{0.978}$		

The selected subset is underlined.
The notations for variables (X_1 to X_6) are as used in Table 1.

Table 3. Direct and indirect effects of contributing characters on seed yield in soybean

	X_1	X_2	X_3	X_4	X_5	X_6	'R' with yield
X_1	$\underline{0.0012}$	0.0038	-0.0175	0.6502	0.0005	-0.0004	0.6378
X_2	0.0009	$\underline{0.0049}$	-0.0214	0.8374	0.0009	-0.0115	0.8112
X_3	0.0006	0.0032	$\underline{-0.0324}$	0.5354	-0.0038	-0.0232	0.4798
X_4	0.0008	0.0042	-0.0180	$\underline{0.9635}$	0.0162	-0.0087	0.9580
X_5	0.0000	0.0000	0.0007	0.0917	$\underline{0.1706}$	-0.0131	0.2499
X_6	0.0000	-0.0005	0.0071	-0.0787	-0.0210	$\underline{0.1067}$	0.0136

Residual = +0.0438
Underlined values show the direct effects
The notations for variables (X_1 to X_6) are as used in Table 1.

NATIONAL RESEARCH CENTER FOR SOYBEAN (ICAR)
Khandwa Road
Indore, Madhya Pradesh 452001
India

245

Field screening of soybean germplasm for resistance to insect pests and diseases

Insect pests and diseases cause severe economic losses to soybean, Glycine max (L) Merrill, and are responsible for low productivity (1.10 t/ha) in India. Several control measures involving costly synthetic pesticides have been recommended but, owing to their indiscriminate use and hazardous effects, it is all the more necessary to part with sole dependence on agrochemicals. In view of rapidly increasing cost of production, it is inevitable to have soybean varieties with resistance/tolerance to the biotic menaces.

Soybean stem flies (Melanagromyza soja and Ophiomyi phaseoli), being internal feeders, are difficult to control chemically, and are responsible for infesting up to 75-90 percent of the crop reducing yield to more than 31 percent (Bhattacharjee, 1976; Gangrade and Singh, 1977). Earlier screening for resistance to stem fly has indicated that no soybean genotype is completely free from this infestation. Categorization on the basis of relative damage has revealed that wild soybean, Glycine soja is highly resistant and line IC 18736 is the least susceptible (Chiang and Talekar, 1980; Chiang, 1984; Kundu and Goswami, 1985). On the disease scenario, bacterial pustule caused by Xanthomonas campestria pv. glycine, is one of the prime diseases, causing 28 percent loss in grain yield (Hartwig and Jhonson, 1953; Khare, 1986).

In order to identify potential sources of resistance/tolerance primarily to stem fly and bacterial pustule, two field screening trials were conducted at National Research Center for Soybean, Indore (M.P.), India, during kharif 1991.

Materials and methods: For insect resistance, 46 pre-identified germplasm lines promising to girdle beetle (Obereopsis brevis) resistance and 12 cultivated varieties including susceptible JS 72-44, were planted in randomized block design with three replications. Each germplasm/variety had three rows of 2m length each. Besides stem fly, observations on other major insects like blue beetle (Cneorane spp.), green semilooper (Chrysodiexis acuta) and leaf folder (Hedylepta indicata) were also recorded

at their peak incidence, i.e. 60 days after germination (DAG), 15 DAG, 60 DAG and 70 DAG respectively. Germplasm/varieties recording less than 25 percent stem fly infestation, 0-2 blue beetle per m row length, 0-4 green semilooper larvae per m row length and 0-5 leaf folder larvae per m row length, were selected as possible sources of resistance.

For disease resistance, 45 out of 111 germplasm lines identified for bacterial pustule resistance under natural conditions (Shukla and Prabhaker, 1991), were screened in field under artificial epiphytotic conditions during kharif 1991. Each genotype was sown in 3.75 m row length and replicated twice. Punjab-1, a highly susceptible cultivar, was used to form the infester rows. Bacterial suspension from highly infected Punjab-1 leaves was prepared (concentration 1000 cells/ml water) and sprayed twice on 30 days old crop at an interval of 24 hours. Disease intensity was recorded after 30 days of inoculation on 0-5 scale (Singh and Jain, 1988). Disease index was calculated on the basis of 25 leaves randomly selected from upper, middle and lower portion of the plants. Categorization was done as follows: -Immune (I) - O, Resistant (R) - 1 to 5, Moderately resistant (MR) - 6 to 20, Moderately susceptible (MS)- 21 to 50, and Susceptible (S)- 51 and above. Besides bacterial pustule, observations on pod blight (Colletotrichum dematium f.sp. truncatum) and leaf spot (Myrothecium roridum), were also recorded and the genotypes were catagorized according to the disease reaction as follows:- Pod blight (per cent infected pod number): Immune (I)- 0, Resistent (R) -1 to 10, Moderately resistant (MR)- 11 to 25, Moderately susceptible (MS) - 26 to 50, Susceptible (S) - 51-75, and Highly susceptible (HS) - 76 and above; Leaf spot (percent infected leaf area): Immune (I) - nil, Resistant (R) - very low and small resistant type symptoms, Traces (T) - 1 to 5, Light (L) - 6 to 15, Moderate (M) - 16 to 25, Susceptible (S) - 26 to 75, and Highly susceptible (HS) - 76 and above, based on visual observations.

Experimental results and discussion: Germplasm lines and varieties showing resistance to two or more insect species have been selected and compared with the susceptible check JS 72-44 in table-1. A black seeded line TGx 540-23E, which exhibited resistance to all the test insects, could be of immense value to the breeder for incorporating this character in a high yielding but susceptible cultivar, to make an attractive proposition for commercial production. Lambert and Kilen (1984) have indicated

that the development of cultivars from lines with resistance to several insect species might be possible without screening for each insect species at each selection stage in a breeding program. Yet another black-seeded line, L - 64, besides being resistant to the stem fly, blue beetle and green semilooper, was also found to be a good yielder. It was interesting to note that lines DS - 396, (yellow), Soja savana (yellow), TG x 814 - 26E (yellow) and TG x 855 - 53D (yellow), despite being infested with more than the desired level, gave better yields, thus confirming tolerance to the insects. Lines TG x 568 - 12D (black) and PLSO - 1 (black) might prove to be good sources for incorporating resistance to stem fly in a genotype resistant to green semilooper, like TG x 239 - 30D (yellow).

From disease point of view, 13 accessions were found to be promising sources of resistance to one or the other diseases (Table 2). Out of eight accessions found resistant to bacterial pustule, EC 241412 (black) and PK 442 (greenish yellow), showed resistance to pod blight also, with low level of leaf spot infection. EC 241412 also showed high yield potential. Cultivar MACS 13 (yellow) and line EC 34500 (green) were also found to be good yielders, besides being resistant to bacterial pustule. A yellow-seeded accession, AGS 109 could serve as an ideal donor for resistance to blue beetle, green semilooper and leaf folder, and moderate level of resistance to bacterial pustule and pod blight.

Some of the lines showing resistance to both the insects and the diseases are: - IQ 5186 (yellow) - green semilooper, leaf folder, bacterial pustule; TG x 1073-55E (yellow) - blue beetle, leaf folder, leaf spot; TK - 5 (yellow) - leaf folder, pod blight; L-537 (green) - leaf folder, bacterial pustule, leaf spot.

We hope that the information contained in this report will help in the development of soybean varieties with resistance/tolerance to insect pests and diseases, thereby reducing the use of pesticides, while maintaining good agronomic characteristics.

References

Bhattacharjee, N.S. 1976. Control of soybean agromyza. Entomologist Newsletter 6:36.

Chiang, H.S. 1984. Soybean resistance to stem mining agromyzid beanflies (Diptera: Agromyzidae). Dissertation Abs. Intl, B (Sci & Eng) 44:3296B.

Chiang, H.S. and N.S. Talekar. 1980. Identification of sources of resistance to the beanfly and two other agromyzid flies in soybean and mungbean. J. Econ. Entomol. 73:197-199.

Gangrade, G.A. and O.P. Singh. 1977. Effect of stemfly M. phaseoli (Tryon) on the yield of pods and grain of soybean in India. Zeitschrift fur Angewandte Entomologie. 80:438-441.

Hartwig, E.E. and H.W. Johnson. 1953. Effect of bacterial pustule disease on yield and chemical composition of soybean. Agron. J. 45:22-23.

Khare, M.N. 1986. Proceedings of National Seminar and 17th Annual Workshop of AICRPS held at MACS, Pune. April 22-25. 1986. GBPUA&T, Pantnagar, pp 135-141.

Kundu, G.G. and K. Goswami. 1985. Evaluation of soybean germplasm for resistance to the stemfly O. phaseoli (Try) J. Ent. Res. 9:54-56.

Lambert, L. and T.C. Kilen. 1984. Multiple insect resistance in several soybean genotypes. Crop Sci. 24:887-890.

Singh, R.B. and J.P. Jain. 1988. Screening of soybean germplasm against Xanthomonas campestris pv. glycines. Indian J. Mycol. Pl. Pathol. 18: 282-283.

Shukla, A.K. and Prabhakar. 1991. Sources of resistance against bacterial pustule. Agril. Biol. Res. (In press).

A.N. Sharma
A.K. Shukla

Table 1. Reaction of promising germplasm lines to four insects

Accessions	Stem fly (% infestation)	Blue beetle (Nos./m row)	Green semi-looper (larvae/m row)	Leaf folder (larvae/m row)	Grain yield (g/sq.m)
TB x 540-23E	18.13	1.33	4.00	1.33	49.77
TG x 239-30D	81.45	2.00	3.33	5.00	184.58
L - 64	15.82	2.00	3.67	9.33	210.92
AGS - 109	32.35	2.00	3.33	4.67	184.13
PLSO - 1	19.97	1.33	15.67	2.33	130.21
TG x 568-12D	17.55	1.00	14.33	5.33	25.54
TG x 855-53D	81.50	1.00	1.00	9.67	219.52
TG x 342-325	30.88	1.33	4.33	5.00	181.23
TG x 1073-55E	48.14	1.33	11.00	4.33	192.44
TB x 814-26E	46.50	1.67	1.33	7.00	210.63
PR-13-34-3-B-2	72.17	1.33	16.33	2.00	135.63
Soja savana	80.49	2.00	3.67	6.67	236.96
DS - 396	63.12	1.00	8.00	3.33	310.67
PK - 416	22.28	7.67	2.33	6.67	223.82
IQ - 5186	54.28	3.67	2.67	3.33	200.51
L -599	75.00	3.00	3.33	2.00	142.31
JS 80 - 21	52.52	1.33	7.67	2.33	268.99
Check					
JS 72 - 44	84.00	8.33	9.67	16.33	179.64

Table 2. Reaction of promising germplasm lines to diseases

Accessions	Bacterial pustule	Pod blight	Leaf spot	Grain yield (g/sq.m)
ED 241412	R	R	L	269
EC 251311	R	MR	T	15*
EC 251867	R	MS	L	123
EC 34500	R	MR	T	220
MACS 13	R	MS	L	215
PK 442	R	R	L	97
IQ - 5186	R	MR	L	107
L - 537	R	MS	T	108
L - 450	MR	MR	S	73
AGS - 109	MR	MR	M	173
TG x 1073-55E	MR	MR	T	87
TG x 1073-30E	MR	MS	L	134
TK - 5	S	R	L	47

*Low yield because of poor germination

PUNJAB AGRICULTURAL UNIVERSITY
Department of Plant Breeding
Ludhiana, Punjab
India

24r

Soybean germplasm evaluation: search for low-linolenic lines

Fatty acid composition is an important determinant of soybean oil quality. Linolenic acid has been identified as an unstable component of soybean oil responsible for the undesirable odors and flavors commonly associated with poor oil quality (Dutton et al. 1951; Smouse, 1979; Frankel, 1980). Evans et al (1965) indicated that a low level of linolenic acid would improve stability of oil. The amount of linolenic acid can be decreased by catalytic hydrogenation, but it is expensive. Therefore, the development of soybean cultivars with low level of linolenic acid in the seed oil seems to be a better alternative. This study was an attempt to identify soybean genotypes with low linolenic acid content.

Materials and methods: Sixty genotypes of soybean representing diverse geographic and genetic background comprised the experimental material. These were sown in a randomized, complete block design with three replications each on two sowing dates (end June and first week of July), during the years 1988 and 1989. Four environments were thus created. Each entry was accommodated in a single-row plot of three meter length with row-to-row space of 45 cm. Fatty acid composition was determined by gas liquid chromatography (GLC) following the method of Luddy et al (1968).

Results and discussion: The analysis of variance pooled over environments indicated that variance due to genotypes as well as environments was significant for this fatty acid. Similarly, genotypes x environment interaction was also significant.

The level of linolenic acid among different genotypes varied from 6.13 to 8.66 percent. The lowest level (6.13) was recorded by Himso-1549 followed by PK 564 (6.33) and F 87-3095 (6.48), whereas high level was possessed by SL 116 (8.66) and SL 127 (8.21). Out of sixty genotypes, only three have low linolenic acid content below 6.5.

Thirty-eight had the level below 7.5 and nineteen had above 7.5 percent.

High to moderate heritability values for linolenic acid reported by Liu (1988), Graef et al (1988) and Sharma (1991) indicated the importance of additive gene action for the expression of linolenic acid. Hence, simple selection schemes may be helpful to improve the soybean genotypes for low levels of linolenic acid in order to have good quality soybean oil by using these genotypes as sources of low level of linolenic acid content.

Burton, Wilson and Brim (1983) suggested selection for increased oleic acid % in seed oil as oleic acid + linoleic acid are negetively correlated with each other (Liu, 1988 and Shanna, 1991). Thus selection for increased oleic acid may be effective in reducing linolenic acid content. Since variation in soybean genotypes for oleic acid was more than linolenic acid, indirect selection for increased oleic acid content will reduce the linolenic acid content in the resultant population.

References

Burton, J.W., R.F. Wilson, and C.A. Brim. 1983. Recurrent Selection in soybean. IV. Selection for increased oleic acid percentage in seed oil. Crop. 23:744- 747.

Dutton, H.J., C.R. Lancaster, C.D. Evans, and I.C. Cowan. 1951. The flavor problem of soybean oil. VIII. Linolenic acid. J. Am. Oil Chem. Soc. 28:115-118.

Evans, C.D., H.A. Mosee, B.C. McConnel, C.J. Cowan, J.L. Carter, and F.I. Collins. 1965. Flavor evaluation of natural soybean oils of high and low linolenic content. J. Am. Oil Chem. Soc. 42:736-738.

Frankel, E.W. 1980. Soybean oil flavor stability p.229-244. In D.R. Erickson, E.H. Pryde, O.L. Brackke, T.L. Mounts, and R.A. Faib (ed). Handbook of soy oil processions and utilization. Am. Soybean Assoc., St. Louis, MD.

Graef, G.L., W.R. Fehr, L.A. Miller, E.G. Hammond, and S.R. Cianzio. 1988. Inheritance of fatty acid composition in a soybean mutant with low-linolenic acid. Crop 28:55-58.

Liu, K.H., 1988. Analysis of combining ability and heritability of protein oil and other components of F2 of soybean. Acta Agronomica Sinica. 14: 303-309.

Luddy, F.E., R.A. Balford, S.F. Herb, and M. Pal. 1968. A rapid and quantitative procedure for the production of methyl esters of butter oil and other fats. J. Am. Oil Chem. Soc. 45:549-552.

Sharma, S.R. 1991. Genetic analysis for yield attributes and quality traits in soybean {Glycine max (L) Merr} PhD Thesis, PAU, Ludhiana.

Smouse, T.H., 1979. A review of soybean oil reversion flavor. J.Am. Oil Chem. Soc. 56:747A-750A.

S.R. Sharma
P.S. Phul
K.L. Ahuja

COMENIUS UNIVERSITY
Faculty of Sciences
Bratislava, Slovakia

Slovakia

245

Variance analysis of some soybean quantitative traits in M_2 generation after laser units and ethylenimine treatment

The knowledge of quantitative traits reaction after environmental factors treatment can serve as an appropriate system for evaluation of environmental factors influence. This system of polygenes which determine quantitative traits seems to be very sensitive to environmental factors influence. Therefore, precise analysis of variability after environmental factors influence could be useful not only for scientists dealing with mutagenesis but even for breeders, for practical utilization.

Material and method: Two varieties of dry seeds, AIDA and NADNEPRJANSKAJA, were treated by helium neon laser units during 300 sec. and EI (0.025 and 0.05%) during 24 hours at 20° C. LU were used before EI. M_1 generation was sown in the field in row spacing between rows 35 cm. and in rows 5 cm. M_2 generation was sown in row spacing between rows 20 cm. and in rows 3 cm., 150 plants of each variant treated and check, were statistically analyzed for 12 quantitative traits.

Results and discussion: Yield components for soybeans were determined by many authors: Weatherspoon and Wentz (1934); Woodworth (1932); and Grafius (1964). Upadhyaya and Singh (1979) discovered that various doses of irradiation had a significant influence on the number of seeds per pod, and in a few cases, even on the number of pods per plant. No influence of irradiation on the height of plants occurred. According to Patirana (1980), the most stable quantitative traits were the height of plants, number of nodes on the main stem, length of internodes, number of seeds per pod, and number of pods per node. On the contrary, the most variable traits which were observed were seed weight per plant and number of branches. Gridnev (1979) observed that nitrosomethyl urea and nitrosoethyl urea caused a decrease in plants' height and yields, and an increase of vegetative period duration. Dwiwedy and Pandey (1981), and Ala (1981; 1982) acquired many various mutants

with significantly higher values of quantitative traits which can be considered as appropriate donors in breeding programs.

Variability evaluation in varieties Aida and Nadneprjanskaja after laser units and ethylenimine treatment showed that the influence of both factors was significant on variability in all quantitative traits in M_2 generation. The proportion of varieties in variability was non significant in quantitative traits: number of nodes on main stem, number of pods on main stem, number of nodes on branches, number of pods on branches, number of seeds on branches, and seeds' weight on branches (Table 1). Testing of homogeneity groups showed that, in traits: number of branches, the number of seeds on the main stem and seed weight on main stem of both varieties were non homogenous (Table 2). The highest homogeneity groups among mutagen concentrations occured in number of nodes on branches and sterility on branches. The lowest homogeneity was observed in groups in number of nodes and number of branches.

Table 1. Analysis of variance for 12 quantitative traits

Source of Variation	NB	NSN	NNMS	NPMS	NNB	NPB
Varieties	0.0000	0.0130	0.0722	0.3922	0.1109	0.0588
Mutagens	0.0000	0.0000	0.0000	0.0000	0.0002	0.0002
Interaction	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Significance - varieties	++	+	-	-	-	-
Significance - mutagens	++	++	++	++	++	++
Significance - interaction	++	++	++	++	++	++

Source of variation	NSMS	SMS	NSB	SB	WSMS	WSB
Varieties	0.0000	0.0157	0.8689	0.0261	0.0000	0.9871
Mutagens	0.0000	0.0000	0.0004	0.0000	0.0000	0.0001
Interaction	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Significance - varieties	++	+	-	+	++	-
Significance - mutagens	++	++	++	++	++	++
Significance - interaction	++	++	++	++	++	++

NSN = number of sterile nodes
NB = number of branches
NPMS = number of pods on the main stem
NNMS = number of fertile nodes on the main stem
NPB = number of pods on branches
NNB = number of nodes on branches
NSMS = number of seeds on the main stem
SMS = sterility on the main stem
NSB = number of seeds on the branches
SB = sterility on the branches
WSMS = weight of seeds on the main stem
WSB = weight of seed on the branches

Table 2. Multiple range analysis for quantitative traits by varieties and homogenity of groups

NSB	NB	NNMS	NPMS	NNB	NPB	NSMS	SMS	NSB	SB	WSMS	WSB
A *	A *	N *	N *	A *	A *	N *	N *	N *	A *	N *	N *
N *	N *	A *	A *	N *	N *	A *	A *	A *	N *	A *	A *

A = Aida variety N = Nadneprjanskaja variety

NSN = number of sterile nodes
NB = number of branches
NPMS = number of pods on the main stem
NNMS = number of fertile nodes on the main stem
NPB = number of pods on branches
NNB = number of nodes on branches
NSMS = number of seeds on the main stem
SMS = sterility on the main stem
NSB = number of seeds on the branches
SB = sterility on the branches
WSMS = weight of seeds on the main stem
WSB = weight of seed on the branches

Table 3. Multiple range analysis for quantitative traits by mutagens and homogeneity of groups

NSN		NB		NNMS		NPMS		NNB		NPB	
L^+E^-	*	L^-E^-	*	L^-E^-	*	L^+E^1	*	L^-E^2	*	L^-E^2	*
L^-E^1	**	L^+E^2	**	L^+E^1	*	L^-E^-	**	L^-E^-	*	L^-E^-	**
L^+E^-	*	L^-E^1	***	L^+E^2	**	L^-E^1	***	L^+E^-	*	L^+E^-	***
L^-E^-	*	L^+E^-	***	L^-E^2	**	L^+E^2	**	L^-E^2	**	L^-E^1	***
L^+E^2	*	L^+E^1	**	L^+E^-	**	L^+E^-	*	L^+E^2	**	L^+E^2	**
L^-E^2	*	L^-E^2	*	L^-E^1	*	L^-E^2	*	L^+E^1	*	L^+E^1	*

NSMS		SMS		NSB		SB		WSMS		WSB	
L^-E^-	*	L^-E^-	*	L^-E^2	*	L^-E^2	*	L^-E^-	*	L^+E^-	*
L^-E^2	**	L^+E^2	*	L^-E^-	**	L^-E^1	*	L^-E^2	*	L^+E^-	*
L^+E^1	**	L^-E^2	**	L^+E^-	***	L^-E^-	*	L^+E^1	**	L^+E^-	**
L^+E^2	**	L^-E^1	**	L^+E^2	***	L^+E^2	*	L^+E^2	***	L^+E^-	***
L^+E^-	**	L^+E^1	*	L^+E^1	**	L^+E^-	*	L^+E^-	*	L^+E^1	**
L^-E^1	*	L^+E^-	*	L^-E^1	*	L^+E^1	*	L^-E^1	*	L^-E^1	*

L^- = LU was not used
L^+ = LU was used according to material and method mentioned above
EI^1 = EI was used in 0.025% concentration
EI^2 = EI was used in 0.05% concentration

References

Ala, V. S. 1981. The influence of chemical mutagens on variability of yields components in soybean. Nauc. - techn. bjul. Vseros. NII soji, Nr. 30-31, 77-84, (Russ.)

Ala, V. S. 1982. The variability of yield components after repetitive treatment of chemical mutagens. Selekcija i agrotechn. soji, Novosibirsk, 39-43, (Russ.)

Dwiwedy, A. K., M. P. Pandey. 1981. Evaluation of induced quantitative mutants in soybean. Indian J. Agr. Sci., 51, Nr. 10, 715-718.

Gridnev, A. K. 1979. The sensitivity of various soybean varieties to mutagens. Bjul. NTI po masl. kulturam, Nr. 3, 10-13, (Russ.)

Grafius, J. E. 1964. A geometry for plant breeding. Crop Sci., 4, 241-246,

Guzov, J. L., R. Patirana. 1981. The relation of seeds production and production important traits in various types of variability. Izv. AN SSSR. Ser. Biol. Nr. 1, 66-76, (Russ.)

Weatherspoon, J. H., J. B. Wentz. 1934. A statistical analysis of yield factors in soybeans. Journal Am Soc. of Agric., 26, 524-531.

Woodworth, C. M. 1932. Genetics and breeding in the improvement of the soybean. Ill. Agr. Exp. Sta. Bul. 384.

Upadhyaya, H. D., B. B. Singh. 1979. Induced variability in soybean. Indian J. Genetics & Plant Breeding, 39, Nr. 2.

Svetozar Plesnik

COMENIUS UNIVERSITY
Fac. of Sciences
Bratislava, Slovakia
and
RESEARCH INSTITUTE OF PLANT PRODUCTION
Piestany, Slovakia

245

Aminoacids composition analysis in selected mutants of soybean

Soybean proteins are considered as one of the most important components of not only human nutrition but even utilization in animal production. Nutritive value of soybean proteins is based on the ability of proteins to provide aminoacid composition in concentrations suitable for living organisms. The composition and relations among aminoacids is important to know from breeding point of view where the knowledge enables breeders to orient their breeding project to desired direction. For this reason, the experiments with selected mutants at the Research Institute of Plant Production in Piestany were carried out.

Materials and methods: In experiments, 12 soybean mutants selected from the variety, Nadneprjanskaja, after Laser Emission and Ethylenimine treatment and the variety Nadneprjanskaja were used. Mutants were selected according to various qualitative mutations (leaves shape, pubescence color, vegetation period duration). Mutants and the variety Nadneprja in fields in 1991 and 1992 were grown. Dry seeds for aminoacids analysis were used. First, proteins hydrolysis in 6M of hydrochloric acid at the temperature 140°C during four hours was carried out. Sixteen essential aminoacids asparagine, threonine, serine, glutamine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tryosine, phenylalanine, histidine, lysine and arginine in hydrolysate cycle (sodium - citrate cycle) were determined by aminoacids analyzer (AAA T339 [Mikrotechna Prague]). Carbon, Hydrogen and Nitrogen were determined by dry combustion method by analyzer CHN - 600 (LECO, Corp. St. Joseph, MI, U.S.A.)

Results and discussion: The average contents of aminoacids with variability components in Table 1 are presented. Many papers are dealing with aminoacid composition in soybean (Caldwell et al. (1973), Zlamal (1981), Karlubik (1980), Kelly (1975), Pavlova et al. (1981), Gavriljuk et al. (1981), Vladova (1988), Aliev (1990), Scegorec (1987), Ford (1979). Aminoacid contents presented in particular papers are rather different in dependence on selected soybean varieties and lines, growing area and growing year. In our experiments, the most variable aminoacids in 1991 and 1992 were proline, lysine and arginine. It is commonly known that plant proteins are deficient in one or more aminoacids, and in pulses there are sulfurous aminoacids. Methionine is considered as the limiting aminoacid. The lack of methionine content is often accompanied with the lack of other aminoacids, the most often being threonine and tryptophan (Zlamal, 1981).

Zlamal (1981) stated that the methionine content had not been genetically correlated with any other essential aminoacids. However, phenotypical correlations of methionine with other aminoacids, except lysine and threonine, were significant. In our experiments with mutants no significant phenotypical correlations between methionine and other aminoacids occurred. Similarly, tryrosine was not significantly correlated with other aminoacids. On the contrary, serine, glutamine, glycine, and alanine were significantly correlated with the majority of aminoacids (Table 2).

References

Aliev, D. A., Gusejnov, S. I., 1990. Aminoacids composition of seeds and protein and possibilities of its utilization in selection of quality increase of soybean yields. Vestn. s.-ch. nauky, Baku, Nr.2, 13-18, [Russ.].

Caldwell, B. E., Howell, R. W., Judd, R. W., Johnson, H. W., 1973. Soybeans: Improvement, Production, and Uses. Madison, Wisconsin, USA.

Foard, D. E., Hwang, D. L., Pao, W. L., Wong-Huang, W. O., Yang, W. K., 1979. Radioimunoassay of soybean proteins having high methionine, high half cystine content. Seed Protein Improv. Cereals and Grain Legumes. Proc. Int. Symp. Neuherberg, Vol. 2, Vienna, 430.

Gavriljuk, I. P., Zajceva, L. N., Teleuca, A. S., Cernenok, A. P., 1982. Immunochemical analysis in evaluation of soybean protein quality. Tr. po prikl. botan. genet. i selekciii. VNII rastenievodst., 70, Nr. 2, 89-102, [Russ.].

Karlubik, M., 1980. Aminoacids composition of proteins of some European and American soybean varieties. Polnohospodarstvo, 26, Nr. 7, 628-636, [Slov.].

Kelly, I. D., Bliss, F. A., 1975. Heritability estimates of percentage seed protein and available methionine and correlations with yield in dry beans. Crop Sci., 15, Nr. 6.

Pavlova, L. S., Sajanova, V. V., Sirokova, E. P., Drozdov, V. V., 1980. The study of relation between activity of Kunitz trypsine inhibitor and other qualitative characters in soybean. Genet. probl. zagraznjenja okruz. sredy na terit. Mold. SSR, Tez, dokl., Kisinev, 58-59, [Russ.].

Scegorec, O. V., 1987. Aminoacids composition and biologically valuable soybean proteins in dependence on pre emergent treatment by physical factors. Puti uvelicsenja zern. kultur v Amur. obl., Blagovescensk, 19-24, [Russ.].

Vladova, R., 1988. Soybean storage proteins. Genetika i selekcijz, Nr. 4, 373.

Zlamal, P., Marek, V., Genetic correlations of essential aminoacids. Genetika a slechteni, 17, 2, 88 - 95 [Czech].

Svetozar Plesnik

Frantisek Debre

Igor Mihalovic

Table 1. Carbon, hydrogen, nitrogen and aminoacids content in 12 mutants and Nadneprjanskaja variety in 1991 - 1992

1991	C	H	N	ASP	TRE	SER	GLU	PRO	GLY	ALA
avg.	50.12	7.888	5.424	5.020	1.838	2.075	7.663	2.819	1.862	1.933
st.dev.	0.656	0.111	0.401	0.519	0.136	0.175	0.848	0.460	0.173	0.159
max.	51.33	8.07	6.29	5.74	2.03	2.30	8.82	3.66	2.12	2.21
min.	49.11	7.65	4.9	4.36	1.63	1.83	6.50	2.06	1.60	1.75
1992										
avg.	50.35	7.882	6.028	5.886	2.047	2.341	8.906	4.845	2.017	2.145
st.dev.	0.628	0.121	0.200	0.560	0.146	0.154	0.927	2.538	0.129	0.178
max.	51.16	8.08	6.38	6.91	2.36	2.60	7.35	8.81	2.23	2.50
min.	48.85	7.65	5.76	5.27	1.85	2.05	10.35	2.23	1.75	1.84

1991	VAL	MET	ILEU	LEU	TYR	PHE	HIS	LYZ	ARG	D.M.
avg.	2.200	0.687	1.967	3.339	1.963	2.325	1.408	2.825	3.223	96.08
st.dev.	0.195	0.097	0.211	0.319	0.455	0.340	0.159	0.499	0.605	0.338
max.	2,62	0.76	2.40	3.99	2.70	2.98	1.72	3.68	4.43	96.27
min.	1.89	0.43	1.57	2.65	1.25	1.77	1.10	1.94	2.15	96.59
1992										
avg.	2.506	0.474	2.113	3.612	1.913	2.654	1.568	3.503	4.312	95.77
st.dev.	0.201	0.107	0.198	0.251	0.146	0.187	0.129	0.564	0.686	0.422
max.	2.79	0.65	2.37	4.00	2.16	2.97	1.80	4.45	5.23	96.12
min.	2.10	0.26	1.64	3.16	1.64	2.32	1.36	2.54	3.06	94.64

D.M. = dry matter

Table 2. Correlation coefficients among essential amino acids in 12 mutants and Nadneprjanskaja variety of soybean

	ASP	TRE	SER	GLU	PRO	GLY	ALA	VAL	MET	ILEU	LEU	TYR	PHE	HIS	LYZ
TRE	0.92														
SER	0.95	0.94													
GLU	0.97	0.92	0.96												
PRO	0.71	0.74	0.61	0.66											
GLY	0.89	0.89	0.93	0.91	0.52										
ALA	0.94	0.87	0.92	0.94	0.58	0.95									
VAL	0.76	0.69	0.81	0.78	0.46	0.73	0.74								
MET	-0.21	-0.21	-0.26	-0.16	0.14	-0.25	-0.20	-0.16							
ILEU	0.46	0.42	0.60	0.51	0.15	0.55	0.50	0.85	-0.08						
LEU	0.55	0.49	0.66	0.57	0.14	0.66	0.62	0.85	-0.22	0.92					
TYR	-0.11	0.05	0.05	-0.04	-0.21	0.04	-0.09	0.16	0.24	0.25	0.22				
PHE	0.56	0.54	0.67	0.56	0.27	0.65	0.58	0.87	-0.35	0.90	0.89	0.15			
HIS	0.56	0.52	0.66	0.57	0.24	0.61	0.56	0.89	-0.24	0.90	0.85	0.25	0.91		
LYZ	0.60	0.61	0.67	0.59	0.56	0.58	0.59	0.76	-0.12	0.70	0.67	0.05	0.76	0.71	
ARG	0.71	0.72	0.77	0.71	0.55	0.70	0.72	0.82	-0.18	0.73	0.76	0.09	0.75	0.77	0.88

CHIANG MAI FIELD CROPS RESEARCH CENTER
Tambon Nonghan
Sansai District
Chiang Mia 50290
Thailand

Thailand

245

Inheritance of soybean resistance to purple seed stain

Soybean [Glycine max (L.) Merrill] infected with purple seed stain disease caused by Cercospora kikuchii (Matsumoto & Tomoyasu) Gardner has been known to exist in Thailand since 1974 (Poonpolgul, 1977). Nowadays, two of seven recommended soybean cultivars (Sukhothia I and Chiang Mai 60) are susceptible to this disease particularly when grown in early wet season. Even though this disease rarely reduces grain yield of soybean, heavy infection reduces seed germinability. Discoloration on the seed coat area greater than 50% averaged 17 to 44% lower seed germination than seed free from purple stain tested in sand benches (Poonpogul and Srisombun, 1987). Heritabilities for disease incidence were from medium (Okabe et al., 1990) to high (Wilcox et al., 1975). The aim of this study was to determine the number of genes controlling the resistance of purple seed stain disease.

A cross was made between SJ.2 (resistant cv) and Chiang Mai 60 (susceptible cv) at Sisamrong Field Crops Experiment Station, Sukhothai in wet season 1991. F1 seeds were sown in dry season (Jan-April) and F2 plants were grown in early wet season (May-Aug) when the environment was favorable for disease infection under natural conditions. The number of infected plants, based on discolored symptom on the seeds, were counted after harvesting at R8 growth stage (Fehr and Caviness, 1977) and were classified into two groups. The groups included plants with and without purple seed stain on seed coat. All random plants of the resistant parent showed no symptom of discoloration on the seeds whereas 75% of random plants of the susceptible parent had the purple seeds (Table 1). The F2 segregation gave a good fit to the expected 3:1 ratio suggesting that the resistance of purple seed stain from this cross of soybean cultivars examined under natural infection conditions was monogenetically inherited.

Wilcox et al. (1975) reported that heritability estimates for incidence of purple seed

stain disease was high in F2 generation. There was the possibility of effective selection for resistance to purple seed stain (Okabe et al., 1990). However, selections based on low incidence of infection gave less seed yields than high incidence of infection since the growing period of selected soybean lines was short. In soybean breeding program for purple seed stain resistance, it would be successful when selection of the resistant plants is made as early as in F2 generation from crosses between soybean parents having similar maturity.

Table 1. Reaction of parents and their F2 progeny to soybean purple seed stain disease.

Genotype	Number of plants		Purple seed stain (%)	X^2 probability of fit to expected ratio
	NPS	PS		
SJ.2	20	0	-	
Chiang Mai 60	5	15	0.9-5.1	
F2	74	23	0.6-4.9	0.30 - 0.5 for 3:1

NPS = Nonpurple stain on seed coat
PS = Purple stain on seed coat

References

Fehr, W.R. and C.E. Caviness. 1977. Stages of soybean development. Special report 80. Iowa State University of Science and Technology, Iowa.

Okabe, N., K. Sasaki, and K. Igita. 1990. Varietal difference in resistance to purple seed stain of soybean and the method of selection for the resistance. JARQ 23:163-169.

Poonpolgul, S. 1977. Survey of soybean diseases and studies on soybean downy mildew. Master of Science Thesis (Agriculture). Kasetsart University, Bangkok.

Poonpolgul, S. and S. Srisombun. 1987. The problem of soybean production in early wet season and purple seed stain disease. Kehakaset 11:41-43 (In Thai).

Wilcox, J.R., F.A. Laviolette and R.J. Martin. 1975. Heritability of purple seed stain resistance in soybeans. Crop Sci 15:525-526.

Somsak Srisombun
Permsak Supapornhemin

UNIVERSITY OF ILLINOIS
Department of Agronomy
Urbana-Champaign

United States

Inheritance of brown stem rot resistance in PI 473685D

Introduction: Brown stem rot caused by *Phialophora gregata* (Allington and Chamberlain) W. Gams, has been reported throughout the midwestern and southeastern United States. *P. gregata* is a soil born pathogen that enters soybean through main and lateral roots (Allington and Chamberlain, 1944). During environmental conditions favorable to *P. gregata* growth, conidia and mycelium spread throughout the plant causing browning of the vascular system. Infected plants will develop interveinal leaf necrosis followed by a wilting and drying of leaves (Abel, 1977). Infection of *P. gregata* can result in soybean yield reduction of 12% to 38% (Gray, 1972; and Sebastian et al., 1986).

Currently, three dominant genes conferring resistance to *P. gregata* have been reported. The *Rbs1* gene was identified in the breeding line L78-4094 (Hanson et al., 1988; Sebastian and Nickell, 1985), which derives its resistance to brown stem rot from PI 84946-2. Sebastian and Nickell (1985) suggested that PI 84946-2 contains the *Rbs1* and possibly a second gene for brown stem rot resistance. The *Rbs2* gene (Hanson et al., 1988) and *Rbs3* gene (Willmot and Nickell, 1989) were identified in PI 437833 and PI 437970, respectively. Recently, Waller et al. (1991), suggested that polygenic resistance occurs in Asgrow A3733, which does not derive its resistance to brown stem rot from known sources of resistance. At the present time, all publicly released cultivars with brown stem rot resistance derive their resistance from PI 84946-2. Since variability in pathogenicity of *P. gregata* isolates had been reported (Willmot, 1988), additional sources of resistance would be beneficial to breeding programs.

In 1989, Nelson et al. identified that PI 437685D was resistant to *P. gregata* at Hancock, Wisconsin. Willmot et al., (1989) determined that PI 437685D contained a single dominant gene that was not allelic to the *Rbs1* and

F_1 plants, 288 to 339 F_2 plants and a set of standard differentials were evaluated against relatively homogeneous populations of SCN Race 3 isolate as described by Rao-Arelli and Anand (1988). The inoculation techniques used in this study were already reported (Rao-Arelli et al., 1991). Thirty days after inoculation plant roots were washed and the dislodged white females were counted using a stereo microscope.

In previous genetic studies of SCN resistance, plants have been classified as either highly resistance or susceptible based on the index of parasitism (IP). Resistance has been defined as <10% IP, following the classification standard established by Golden et al., 1970. In this report, we have defined resistance strictly based on the reaction of resistant parent i.e., range of cysts observed for 10 plants in each of the four different accessions. Thus in each cross, individual F_1 and F_2 plants with reaction for resistance similar to that of their respective parents, which is solely based on range of cysts obtained, were identified resistant. Chi-square analysis was used to test goodness of fit to appropriate genetic hypothesis.

Results and discussion: Means, ranges of cyst counts and variances for F_1 and F_2 plants of the four different crosses obtained in this report are provided in Table 1. The observed resistant vs. susceptible frequencies for F_1 and F_2 plants are also included in the same table, along with hypothesized resistance genes in each of the four resistant parents.

The F_1 hybrids of all four crosses were susceptible to SCN Race 3 isolate. Their means and ranges for all four crosses are below that of susceptible parent, Essex. This indicated incomplete dominance of Essex's susceptibilty over nematode resistance in the resistant parents used in this report. Similar results were already reported (Rao-Arelli et al., 1989).

The F_2 plants in each of the two crosses PI438489B x Essex and PI404166 x Essex, segregated closely to 1R:15S and 9R:55S, respectively. The remaining crosses, PI89772 x Essex and PI209332 x Essex, both segregated closely to 3R:13S in F_2 generation. This indicates that PI438489B has two major recessive genes, and PI494166 has two dominant genes plus one

recessive gene for resistance, respectively. PI89772 and PI209332 both have one dominant and one recessive gene for resistance to SCN Race 3 isolate.

We await data confirming our F_2 results and for confirmation of our values with and without index of parasitism.

References

Caldwell, B. E., C. A. Brim and J. P. Ross. 1960. Inheritance of resistance of soybeans to the cyst nematode, *Heterodera glycines*. Agron. J. 52:635-636.

Golden, A. M., J. M. Epps, R. D. Riggs, L. A. Duclos, J. A. Fox and R. L. Bernard. 1970. Terminology and identity of infraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Dis. Rep. 54:544-546.

Matson, A. L. and L. F. Williams. 1965. Evidence of a fourth gene for resistance to the soybean cyst nematode. Crop Sci. 5:477.

Rao-Arelli, A. P. and S. C. Anand. 1988. Genetic relationships among soybean plant introductions for resistance to Race 3 soybean cyst nematode. Crop Sci. 28:650-652.

Rao-Arelli, A. P., S. C. Anand and G. O. Myers. 1989. Partial dominance of susceptibility in soybean to soybean cyst nematode Races 3, 4 and 5. Crop Sci. 29:1562-1564.

Rao-Arelli, A. P., K. W. Matson and S. C. Anand. 1991. A rapid method for inoculating soybean seedlings with *Heterodera glycines*. Plant Dis. 75:594-597.

Rao-Arelli, A. P., S. C. Anand and J. A. Wrather. 1992. Soybean resistance to soybean cyst nematode Race 3 is conditioned by an additional dominant gene. Crop Sci. 34:862-864.

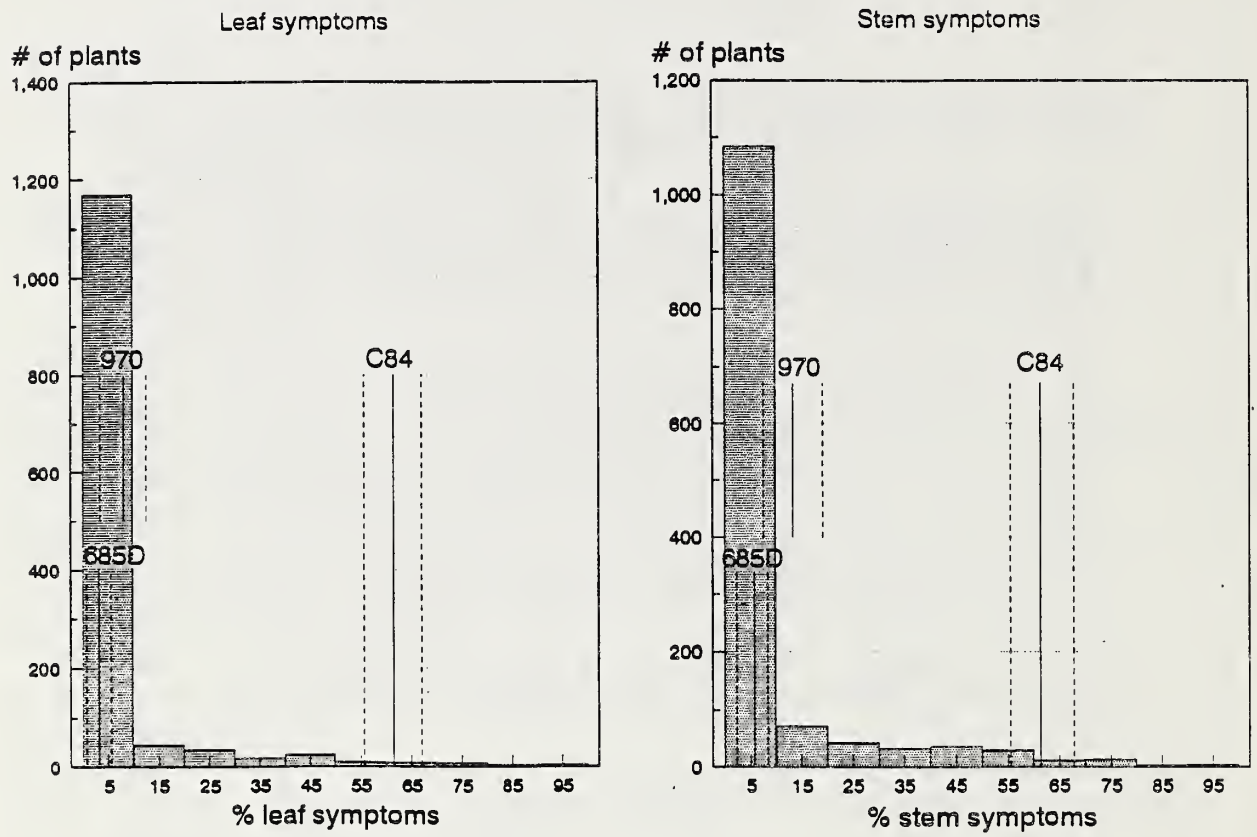
A. P. Rao-Arelli
P. A. Owen
G. U. Berger

Table 1. Results of F_2 and F_3 generations of the cross between PI 437970 x PI 437685D. Plants were classified as resistant or susceptible by the lower 95% confidence limit of symptoms on Century 84.

Symptoms	Gen.†	Checks	Checks mean‡	Checks std. err.§	Res.¶	Seg.#	Sus.††	Ratio tested	Chi-square	p value
			— % —			no.				
Leaf	F_2				59		0	15:1	2.94	0.09
		Century 84	72.6	6.3						
		PI 437970	1.8	1.2						
		PI 437685D	0.0	0.0						
	$F_{2:3}$ families				40	0	0	11:4:1	18.18	0.00
	F_3				1300		32	55:9	149.85	0.00
		Century 84	61.2	2.8						
		PI 437970	8.1	2.3						
		PI 437685D	3.0	1.1						
Stem	F_2				59		0	15:1	2.94	0.09
		Century 84	86.1	5.5						
		PI 437970	7.1	2.8						
		PI 437685D	4.4	4.4						
	$F_{2:3}$ families				40	0	0	11:4:1	18.18	0.00
	F_3				1284		48	55:9	103.57	0.00
		Century 84	61.5	3.0						
		PI 437970	13.6	2.8						
		PI 437685D	5.3	1.7						

† Generation
‡ Mean of symptoms observed.
§ Standard error of symptoms observed.
¶ Resistant genotypes and families that are resistant or segregating 15:1 (resistant:susceptible).
# Families segregating 3:1 (resistant:susceptible).
†† Susceptible genotypes and families that are susceptible.

Fig. 1. Histograms of F_3 plants for leaf and stem symptoms from the cross of PI 437970 x PI 437685D. Solid lines are the mean value and the dotted lines are the 95% confidence interval. The symbols C84, 970, and 685D represent Century 84, PI 437970, and PI 437685D, respectively.



S. R. Eathington
C. D. Nickell

UNIVERSITY OF ILLINOIS
Department of Agronomy
Urbana, IL 61801

245

Elemental content of callus from five soybean genotypes grown on a conventional and a low iron medium.

Introduction: Over the last decade a substantial amount of research has been conducted evaluating the response of soybean cell cultures to low iron. Sain and Johnson (1984) reported that the Fe-inefficient genotype PI 54.619 cell suspension cultures required more FeEDTA than Fe-efficient 'Hawkeye' for normal growth. These same authors (Sain and Johnson, 1986) also demonstrated that, compared to 'Hawkeye', PI 54.619 had a diminished ability for iron uptake when grown under iron deficient conditions. In a later study, Cornett and Johnson (1991) compared the ferric (Fe^{3+}) reducing capacity of intact soybean roots to suspension cultures and found that, unlike intact roots, Fe^{3+} reduction in the suspension culture occurred regardless of the iron concentration in the nutrient medium. More recently, friable callus has also been successfully employed in screening techniques to identify Fe-efficient soybean genotypes (Stephens et al., 1991; Graham et al., 1992).

One area that has not been investigated is the elemental composition of friable callus grown on normal 4MSII and low iron media. Therefore, studies were initiated to compare the levels of six elements (P, K, Ca, Mn, Fe, and Zn) of five soybean genotypes with different levels of resistance to Fe-deficiency, grown on the conventional 4MSII medium and on a low iron medium.

Materials and methods: The following five genotypes with varying levels of Fe-efficiency were selected for this study: germplasm line 'A14' and 'Burlison' comprised the Fe-efficient class; cultivar 'Jack' constituted the intermediate class, while cultivars 'A3205' and 'BSR 201' represented the Fe-inefficient class. Callus of each genotype was initiated as described by Barwale et al. (1986). Briefly, seed of each genotype was surface sterilized in 20% Clorox v/v and placed in 25 x 100 mm glass tubes containing MS

(Murassige and Skoog, 1962) basal salts without growth regulators. After 12 days, epicotyl sections were removed and placed onto 4MSII (Barwale et al., 1986) medium for callus formation.

After three weeks, five 40-mg callus pieces of the five genotypes were placed onto a 4MSII medium and a low iron medium, which contained 10μM Fe (Stephens et al., 1991). Since a large amount of tissue is required for the elemental analysis there were five plates of each genotype per replication, with two replications being analyzed. After 28 days, callus was removed, weighed, and placed in a 60°C oven for approximately 48 hours. Dried callus was then ground through a 1 mm mesh, weighed, and placed in digestion tubes. Samples were then digested using a modified Bremner and Mulvaney (1983) salicylic acid-thiosulfate digestion. Elements P, Ca, Zn, Fe, K, and Mn were then analyzed using a Perkin-Elmer Model P2000 Inductively Coupled argon Plasma emission sequential spectrophotometer (ICP).

Results and discussion: Highly significant differences in growth on the low iron medium in comparison with that on the control medium were observed among the five genotypes. The two Fe-efficient genotypes ('Burlison' and 'A14') averaged 28.2 percent of the control weight, whereas the two Fe-inefficient genotypes ('BSR 201' and 'A3205') averaged 14.2 percent of the control. Fe-intermediate 'Jack' was between these two groups (19.6 percent of the control).

Nutrient content of the five soybean genotypes on the control 4MSII medium and the low iron medium differed significantly (Table1). On the control 4MSII medium, Fe-efficient 'A14' had the highest amount of Fe, P, K, and Mn, whereas Fe-inefficient 'A3205' had the lowest amount of Fe, Ca, Zn, and Mn. It is interesting to note the high concentration of Fe in 'A14', since Fe was not limiting in the control medium. This may be a consequence of the increased ability of this genotype to reduce iron. This result also indicates that iron reduction at the cellular level occurs regardless of the iron concentration of the medium, as demonstrated by Cornett and Johnson, (1991). On the low iron medium, significant differences among the five genotypes were only detected

for P, Zn, and Mn concentrations. Unlike the 4MSII medium, 'A14' had the lowest concentration of P and Zn, whereas the Fe-inefficient genotype 'BSR 201', had the highest concentration of P and Zn. As expected Fe was significantly lower in all genotypes. Although no significant differences among the genotypes were noted, A14 and Burlison had the highest concentrations of Fe.

In comparing nutrient concentrations of the five genotypes grown on the 4MSII medium to nutrient concentrations of the same genotypes on the low iron media, it is interesting to note the increase in Zn on the low iron concentrations. Because Fe and Zn are ionic antagonists (Ambler et al., 1970), the Fe concentration will have an effect on Zn uptake. Therefore, in the 4MSII media, the high Fe concentration would be inhibitory to Zn uptake and similarly, a low Fe concentration would enhance Zn uptake. Despite the overall increase in Zn uptake on the low Fe media, Fe-inefficient 'BSR201' had twice the amount of Zn as did Fe-efficient 'A14'. Possible explanations for this include: (i) increased capacity of 'A14' to reduce Fe^{3+} limits Zn uptake, or (ii) 'A14' has other mechanisms that reduce Zn uptake. These areas should be addressed in future studies.

References:

Ambler, J. E., J. C. Brown, H. G. Gauch. 1970. Effect of zinc translocation of iron in soybean plants. Plant Physiol. 46:320-323.

Barwale, U. B., H. R. Kerns, J. M. Widholm. 1986. Plant regeneration from callus cultures of several soybean genotypes via embryogenesis and organogenesis. Plants. 67:473-481.

Bremner, J. M., C. S. Mulvaney. 1982. Nitrogen - Total. *In* A. L. Page (ed.) Methods of soil analysis, part 2, Agronomy 9 (2nd edn.):595-624. Am Soc. Agron., Madison, Wis.

Cornett, J. D., G. V. Johnson. 1991. Ferric chelate reduction by suspension culture cells and roots of soybean: A kinetic comparison. Plant and Soil 130:175-180.

Graham, M. J., P. A. Stlephens, J. M. Widholm, C. D. Nickell. 1992. Soybean evaluation for iron deficiency chlorosis using sodium bicarbonate and tissue culture. J. Plant Nutr. 15:1215-1225.

Murashige T., F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Phyusiol. Plant. 15:473-497.

Sain, S. L., G. V. Johnson. 1984. Selection of iron-efficient soybean cell lines using plant cell suspension culture. J. Plant Nutr. 7:389-398.

Sain, S. L., and G. V. Johnson. 1986. Characterization of iron uptake by iron-efficient and iron-inefficient soybeans in cell suspension culture. J. Plant Nutr. 9:729-750.

Stephens, P. A., J. M. Widholm, C. D. Nickell. 1990. Iron deficiency chlorosis evaluation of soybean with tissue culture. Theor. and Appl. Gen. 80:417-420.

M. J. Graham
J. M. Widholm
C. D. Nickell

Table 1. Friable callus element content of five soybean genotypes grown on 4MSII and low iron media.

Genotype	Nutrient Concentration[a]											
	4MSII[b]						Low Iron Media[c]					
	P	Ca	K	Mn	Fe	Zn	P	Ca	K	Mn	Fe	Zn
	ppm											
A14	30.3	32.9	420	4.4	3.2	0.1	31.4	25.6	238	4.0	0.3	1.9
BSR 201	27.4	32.7	388	4.3	1.3	0.1	46.7	29.3	322	3.9	0.1	3.7
Burlison	24.9	24.8	405	4.3	1.5	0.1	42.1	30.2	337	3.8	0.4	2.0
A3205	24.9	27.6	402	3.8	0.8	0.1	32.1	24.6	377	3.3	0.2	3.0
Jack	20.9	27.9	377	3.7	1.0	0.1	43.7	30.5	327	4.6	0.1	2.9
Mean	25.7	29.2	398	4.1	1.6	0.1	39.2	28.1	320	3.9	0.2	2.7
LSD (0.5)	3.7	3.9	39	0.3	1.2	NS	12.8	NS	NS	1.1	NS	1.9

[a] Based on an initial callus sample of 0.5 grams
[b] contains 100 μM Fe^{3+}
[c] contains 10 μM Fe^{3+}

UNIVERSITY OF ILLINOIS
Department of Agronomy
Urbana, IL 61801

247

Detection of hybridization in crosses between *Glycine tomentella* and *G. max* using isozyme markers //

Introduction: Wild perennial Glycine species are diverse morphologically, cytologically and genomically (Singh et al., 1992) and harbor useful traits (Kenworthy, 1989) that would be worth transferring to the cultivated annual soybean [G. max (L.) Merr.] through wide hybridization and backcross procedures. Due to extremely low crossability and the need to employ immature seed-rescue techniques in order to obtain F_1 hybrids, the wild perennial Glycine species have not been exploited in soybean breeding programs. However, recently Singh et al. (1993) obtained (BC_2-BC_4)-derived fertile plants from the soybean and G. tomentella Hayata intersubgeneric hybrids. The purpose of this paper is to provide information about which isozyme systems can be used to detect the presence of G. tomentella chromosomes within crosses between G. tomentella and the soybean.

Materials and methods: The parents and origin of the F_1, amphiploid and BC_1 plants were described by Singh et al. (1990): [(Glycine max cv. Altona, $2n=40$, genome GG) x (G. tomentella, PI 483218, $2n=78$, genome DDEE) F_1, $2n=118$ (H213-2a) genome GGDDEE x soybean cv. Clark 63 BC, (H 562-1), 2n=76 (expected 79) genome GGDE]. The F_1 and BC_1 plants were chromosomally self sterile. Occasionally, an amphiploid plant produced a pod containing one or two seeds.

The methods and procedures used in the isozyme analysis were those described by Menancio (1987). The staining procedures used were according to the methods of Vallejos (1983) and/or Cardy and Beversdorf (1984). Isozymes used in the preliminary screening to determine those suitable as good genetic markers were: Aconitase (ACO), alcohol dehydrogenase (ADH), aldolase (ALD), catalase (CAT), diaphorase (DIA), fumarase (FUM), galactose

dehydrogenase, glucose-6-phosphate dehydrogenase (GPD), glutamate dehydrogenase, glutamate-oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), peroxidase (PX), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (PGD), succinate dehydrogenase (SDH) and superoxide dismutase (SOD).

One of the changes in the procedure include the use of leaf instead of seed material. The choise of leaf material is important. One or two trifoliate levels which are healthy and recently mature were rinsed in distilled water, finely chopped using a razor and further ground according to the procedure noted. All aspects of the grinding were done on ice to minimize loss of enzyme activity. The percentage of starch used in the gels varied between 14% and 15% (w:v) as the resolution and intensity of the banding varied from enzyme to enzyme. The isozyme tests were performed on all crosses and parents.

Results and discussion: After preliminary screening of all the isozymes, six appeared to be potentially useful as markers. They were ALD, MDH, PGD, PGI, PGM, and PX. Each of these produced a banding pattern consisting of a multiple number of bands (2-6) with good banding resolution. After running a series of gels varying the starch content, it was determined from comparison of the banding patterns of the hybrids to the original parents, G. max cv. Altona, G. max cv. Clark 63 and G. tomentella, that the isozymes ALD, PGD, and PX were not useful in this study since they showed similar banding patterns with all samples tested. However, MDH, PGI and PGM all showed hybrid banding patterns for F_1, amphiploid and BC, plants when compared to the banding patterns of the parents. MDH was best resolved in the 14% (w:v) starch gel, while PGI and PGM were resolved using the 15% (w:v) gel.

In conclusion, we were able to detect intersubgeneric hybrid plants (F_1, amphiploid and BC_1) by utilizing isozyme procedures on leaf tissue. However, extensive isozyme and molecular studies of fertile plants (BC_3, BC_4) need to be conducted to determine whether DNA from G. tomentella has been integrated into the soybean genome.

Acknowledgement: This research was supported by a Jonathan Baldwin Turner undergraduate research project fund from the College of Agriculture, University of Illinois, Urbana. Mr. Metz currently is a graduate student at Cornell University.

References:

Cardy, B. J., W. D. Beversdorf. 1984. A procedure for the starch gel electrophoretic detection of isozymes of soybean (*Glycine max* [L.] Merr.). Department of Crop Science Tech. Bull. 119-8401. Univ. of Guelph, Guelph, Ontario, Canada.

Kenworthy, W. J. 1989. Potential genetic contributions of wild relatives to soybean improvement. p. 883-888. *In* A. J. Pascale (ed.) World Soybean Research Conference IV. Proc., Buenos Aires. 5-9 Mar. 1989. Assoc. Argentina de La Soja, Buenos Aires.

Menancio, D. I. 1987. Isozyme and RFLP studies in the genus *Glycine* Willd. subgenus *Glycine*. Ph.D. University of Illinois, Urbana, IL.

Singh, R. J., K. P. Kollipara, T. Hymowitz. 1992. Genomic relationships among diploid wild perennial species of the genus *Glycine* Willd. subgenus *Glycine* revealed by meiotic chromosome pairing and seed protein electrophoresis. Theor. Appl. Genet. 85:276-282.

Singt, R. J., K. P. Kollipara, and T. Hymowitz. 1993. Backcross (BC_2-BC_4)-derived fertile plants from *Glycine max* (L.) Merr. and *G. tomentella* Hayata intersubgeneric hybrids. Crop Sci. 33 (accepted).

Vallejos, C. E. 1983. Enzyme activity and staining. p. 469-516. *In* S. D. Tanksley and T. J. Orton (eds.) Isozymes in Plant Genetics and Breeding, Part A. Elsevier, Amsterdam.

Timothy D. Metz
Theodore Hymowitz

UNIVERSITY OF ILLINOIS
USDA/ARS
Urbana, IL 61801

245

Genetic analysis of a hypernodulating mutant of soybean

Introduction: Recently, mutagenesis has been used on a number of adapted soybean [Glycine max (L.) Merr.] cultivars to introduce variability in the nodulation phenotype. Gremaud and Harper (1989) characterized three hypernodulating mutants which had the ability to hypernodulate. Carroll et al. (1985) reported that the supernodulating phenotype in nts382 was inherited as a single recessive gene in crosses with the wild-type parent 'Bragg' (Hinson and Hartwig, 1964). Mathews et al. (1990) found similar results in progeny from crosses between the supernodulating mutant nts382 and the wild-type 'Bragg'. The terms supernodulation and hypernodulation are used to describe extensive and intermediate degrees of nodulation, respectively, compared to wild-type plants (Gresshoff and Delves, 1986).

There have been six genes identified that affect nodulation in soybean. Three genes, Rj_2 (Caldwell, 1966), Rj_3 (Vest, 1970), and Rj_4 (Vest and Caldwell, 1972), are responsible for ineffective nodulation with specific strains of [Bradyrhizobium japonicum (Kirchner) Buchanan]. Three genes, rj_1 (Williams and Lynch, 1954) and rj_5 and rj_6 (Pracht et al., 1992), are responsible for nonnodulation. Mathews et al. (1990) evaluated progeny from crosses with a supernodulating mutant and three nonnodulating mutants and reported that the nonnodulation genotype epistatically suppressed the supernodulation genotype. The objectives of this study were to characterize the hypernodulating mutant NOD4, determine the number of genes responsible for hypernodulation, and determine if hypernodulation could overcome the nonnodulation response condition by rj_1 (Williams and Lynch, 1954).

Materials and methods: The hypernodulating line NOD4, was previously selected (Gremaud and Harper, unpublished) from mutagenized seed of the cultivar 'Williams' (Bernard and Lindahl, 1972). To determine the number of

genes responsible for hypernodulation, reciprocal crosses were made between NOD4 and two normally nodulated cultivars 'Chapman' (McBlain et al., 1991) and 'Harosoy 63' (Williams and Bernard, 1964). To determine if the gene(s) responsible for hypernodulation in NOD4 could overcome the nonnodulating phenotype conditioned by $\underline{rj_1}$ (Williams and Lynch, 1954), a nonnodulating near isoline of 'Harosoy' containing $\underline{rj_1}$, L65-1274 (Bernard et al., 1991), was crossed with NOD4. F_1 seed were inoculated prior to planting with a commercial peat-based B. japonicum (Urbana Laboratories, St. Joseph, MO). At 14 d after planting, seedlings were evaluated for nodulation type. The F_1 plants were then space-planted in the greenhouse to produce F_2 seed. The F_2 seed were inoculated and 14 days after emergence the roots were visually classified as either nonnodulating, nodulating, or hypernodulating and transplanted to the field to produce F_3 seed. The F_3 plants from normally nodulated F_2 plants were classified in the greenhouse for nodulation type. The F_3 plants derived from seed of F_2 plants, exhibiting either hypernodulation or nonnodulation, were also evaluated to determine if the F_2 plants were classified correctly.

Results and discussion: The F_1 plants of the crosses made between NOD4 and normally nodulated cultivars, 'Harosoy 63' and 'Chapman', were normally nodulated, indicating that the hypernodulation phenotype exhibited by NOD4 is controlled by a recessive gene. The F_1 plants from the crosses of NOD4 with L65-1274 were nodulated, indicating that the gene(s) responsible for hypernodulation in NOD4 were not allelic to rj_1. All of the F_1 plants had purple flowers and tawny pubescence, therefore all the F_1 plants were derived from successful crosses.

The F_2 plants from crosses between NOD4 and cultivars 'Harosoy 63' and 'Chapman' indicate that a single recessive gene is responsible for hypernodulation in NOD4 (Table 1). The relatively low probability of the reciprocal crosses of NOD4 and 'Harosoy 63' to fit three normal : one hypernodulating model, may have been due to poor root growth and misclassification of hypernodulating types as normal nodulating types. Evaluation of normal nodulated F_2 plants in the F_3 generation classified a few

nonsegregating hypernodulating types, which would support the assumption of misclassification in the F_2. Two gene segregation in crosses between NOD4 and L65-1274 confirms that the gene responsible for hypernodulation in NOD4 is not allelic to rj_1 (Table 1). The segregation of the $F_{2:3}$ families confirms that a single recessive gene in NOD4 is responsible for the hypernodulation phenotype (Table 2).

F_3 plants derived from hypernodulating F_2 plants from the crosses of NOD4 and L65-1274 (data not shown) segregated for nonnodulation and hypernodulation, while some produced all hypernodulating types. This supports the epistatic model proposed in Table 1. Plants that exhibited nonnodulation in the F_2 produce all nonnodulating plants in the F_3 generation which further support the assumption of epistasis. Mathews et al. (1990) selected plants from the F_2 that had above normal nodulation and evaluated them in the F_3 generation. They found that nodulation of F_3 segregants could be classified as one extreme supernodulated : two intermediate supernodulated : one nonnodulated. Evaluation of F_3 plants from hypernodulating F_2 plants from the crosses between NOD4 and L65-1274 indicated that there may be an intermediate class, but variation of hypernodulating plants made it difficult to separate the two classes.

References:

Bernard, R. L., D. A. Lindahl. 1972. Registration of Williams soybean. Crop Sci. 12:716.

Bernard, R. L., R. L. Nelson, C. R. Cremeens. 1991. USDA soybean genetic collection: Isoline collection. Soybean Genet. Newsl. 18:27-57.

Caldwell, B. E. 1966. Inheritance of a strain-specific ineffective nodulation in soybean. Crop Sci. 6:427-428.

Carroll, B. J., D. L. McNeil, P. M. Gresshoff. 1985. A supernodulating and nitrate-tolerant symbiotic (nts) soybean mutant. Plant Physiol. 78:34-40.

Gremaud, M. F., and J. E. Harper. 1989. Selection and initial characterization of partially nitrate tolerant nodulation mutants of soybean. Plant Physiol. 89:169-173.

Gresshoff, P. M., A. C. Delves. 1986. Plant genetic approaches to symbiotic nodulation and nitrogen fixation in legumes. In A. D. Blonstein and P. J. King (eds.) A Genetic Approach to Plant Biochemistry. Plant Gene Research. Springer-Verlag, New York. pp. 159-206.

Hinson, K., E. E. Hartwig. 1964. Bragg and Hardee soybeans. Crop Sci. 4:664.

Mathews, A., B. J. Carroll, P. M. Gresshoff. 1990. The genetic interaction between nonnodulation and supernodulation in soybean: an example of developmental epistasis. Theor. Appl. Genet. 79:125-130.

McBlain, B. A., R. J. Fioritto, S. K. St. Martin, A. Calip-DuBois, A. F. Schmitthenner, R. L. Cooper, R. J. Martin. 1991. Registration of 'Chapman' soybean. Crop Sci. 31:487-488.

Pracht, J. E., C. D. Nickell, J. E. Harper. 1992. $\underline{Rj_5}$ and $\underline{Rj_6}$, genes controlling nodulation in soybean. Crop Sci. (submitted).

Vest, G. 1970. $\underline{Rj_3}$ - A gene conditioning ineffective nodulation in soybean. Crop Sci. 10:34-35.

Vest, G., B. E. Caldwell. 1972. $\underline{Rj_4}$ - A gene conditioning ineffective nodulation in soybean. Crop Sci. 12:692-693.

Williams, L. F., R. L. Bernard. 1964. Hawkeye 63, Harosoy 63, and Chippewa 64 soybeans. Crop Sci. 4:663.

Williams, L. F., D. L. Lynch. 1954. Inheritance of a nonnodulating character in the soybean. Agron. J. 46:28-29.

James E. Pracht
Cecil D. Nickell
James E. Harper

Table 1. Effects of inoculation with *Bradyrhizobium japonicum* on nodulation reactions of F_2 plants from crosses between the hypernodulating NOD4 mutant from Williams and normal nodulating and nonnodulating soybean lines.

Cross	Alleles	Total plants	Observed normal	Observed hypernod	Observed nonnod	Theoretical ratio	X^2	Prob.
		no.						
NOD4 x Harosoy 63†	$\frac{Rj_1 rj_h}{Rj_1 Rj_h}$	345§	273	72	0	3:1	3.1391	0.08
NOD4 x Chapman†	$\frac{Rj_1 rj_h}{Rj_1 Rj_h}$	164§	119	45	0	3:1	0.5203	0.47
NOD4 x L65-1274‡	$\frac{Rj_1 rj_h}{rj_1 Rj_h}$	353§	190	67	96	9:3:4	1.0595	0.59

† Normal nodulating soybean lines.

‡ Nonnodulating soybean line.

§ Combined total of reciprocal crosses from four to five F_1 plants, since no significant difference was found.

Table 2. Effects of inoculation with *Bradyrhizobium japonicum* on nodulation reactions of F_3 families derived from normally nodulated F_2 plants from crosses between the hypernodulating NOD4 line and nodulating Harosoy 63, nodulating Chapman, and nonnodulating L65-1274.

Cross†	(seg/non-seg)	Nodulation seg	Nodulation non seg	X^2	Probability
		no.			
NOD4 x Harosoy 63	2:1	33	14	0.2660	0.61
NOD4 x Chapman	2:1	14	10	0.7500	0.39
NOD4 x L65-1274‡	8:1	43	4	0.3218	0.57

† Combined total of reciprocal crosses from four to five F_1 plants, since no significant difference was found.

‡ L65-1274 is a nonnodulating isoline of Harosoy containing rj_1.

IOWA STATE UNIVERSITY
Department of Agronomy
Ames, IA 50011

Identification of a RAPD marker linked to the RPS_4 *Gene in soybean [Glycine max (L.) Merr.]*

Introduction: Phytophthora root rot in soybean [Glycine max (L.) Merr.], induced by Phytophthora megasperma Drechs. f. sp. glycinea, is one of the most destructive diseases throughout the soybean production areas in the United States and Canada (Athow, 1987). Genetic resistance offers the only viable means of control of this pathogen. One important source of dominant genetic resistance was identified in PI86050 by Athow et al. (1980) and was subsequently designated Rps_4.

Selection for phytophthora resistance is often hindered since it is difficult to ensure uniform exposure to inoculum in the field (Kaufmann et al. 1958). Many breeders use artificial inoculation techniques to screen for phytophthora resistance. Indirect selection for resistance using marker assays could be more reliable or simpler than inoculation tests and offer the possibility of screening breeding materials on a large scale with acceptable costs. Furthermore, molecular markers may facilitate selection and pyramiding of resistance genes into susceptible germplasm. Previous studies have utilized restriction fragment length polymorphism (RFLP) markers to map resistance genes in crop plants (Young et al. 1988; Sarfatti et al. 1989; Diers et al. 1992). More recently, random amplified polymorphic DNA (RAPD) markers have been demonstrated as useful genetic markers for a variety of organisms (Williams et al. 1990; Martin et al. 1991; Michelmore et al. 1990; Paran et al. 1991; Miklas et al. 1993). We have identified a RAPD marker that is linked to the Rps_4 resistance gene using near isogenic lines (NILs).

Materials and methods:

Genetic Material

Williams and a set of six Williams NILs that each have a gene for resistance to phytophthora at a different locus were screened using arbitrary primers with the polymerase chain reaction (PCR) (Operon Technologies Inc., Alameda, CA) to identify DNA polymorphisms. Williams contains the allele conferring susceptibility at all six resistance loci. The parentage of each NIL is summarized in Table 1. The screening of

31 primers against Williams and the six Williams NILs resulted in the identification of a polymorphism between Williams and the NIL containing *Rps*$_4$ (Williams-*Rps*$_4$). Williams-*Rps*$_4$ was developed through six backcrosses using Williams as the recurrent parent and PI86050 as the donor parent. Linkage between *Rps*$_4$ and the polymorphism was tested using a population formed by crossing Williams-*Rps*$_4$ and Williams. This population included 44 $F_{2:3}$ lines and was the same population Diers et al. (1992) originally used to map *Rps*$_4$. Phytophthora resistance was determined by testing 7 to 15 $F_{2:3}$ individuals derived from each F_2 plant using a modified hypocotyl-puncture method (Morgan and Hartwig, 1965) with modifications described by Diers et al. (1992). Disease reactions for individual plants were classified as resistant or susceptible. The genotypes of the F_2 individuals were determined based on the disease reactions of the F_3 progeny. For calculating linkages, the heterozygous and homozygous resistant F_2 plants were not distinguished because it would have required testing too many F_3 plants from each F_2 individual to do this.

RAPD Analysis

The template DNA used for PCR reactions was extracted from young leaves according to Saghai-Maroof et al. (1984) with minor modifications. The DNA was extracted from a bulk of leaves taken from at least five plants for each NIL and five F_3 plants for each F_2 plant. The DNA concentration of each sample was estimated by DNA fluorometry (Hoefer TKO 100, Hoefer Scientific, San Francisco, CA).

The polymerase chain reaction (PCR) procedure reported by Williams et al. (1990) was followed with minor modifications. Amplification was performed with a Perkin Elmer Cetus 9600 thermal cycler. It was necessary to control the thermal ramp which is the time needed for the thermal cycler to go from one target temperature to the next. The machine was allowed to ramp at maximum efficiency unless otherwise specified. Reactions were performed using a temperature profile of: 94°C/4 min; 3 cycles of 94°C/15 sec; 35°C/15 sec, 59 sec thermal ramp to 72°C/75 sec; 40 cycles of 94°C/15 sec, 40°C/15 sec, 59 sec thermal ramp to 72°C/75 sec; 72°C/7 min and an indefinite 4°C soak. The PCR reactions were performed in 25 ul volumes containing 25 ng genomic DNA template and 25 ng of a single decamer primer, 2 units Stoffel Fragment Polymerase (Perkin Elmer Cetus, Norwalk, CT), 1X buffer [10 mM Tris-HCl (pH8.3), 10

mM KCl], 5.0 mM MgCl, and 200 mM each dNTP. Amplification products were resolved by electrophoresis in 1.4% agarose gels containing ethidium bromide, 40 mM Tris-acetate, and 1 mM EDTA (Williams et al. l990).

Results and discussion: The objective of our work was to identify RAPD markers linked to phytophthora disease resistance genes. Primer OPB-11 (5'-GTAGACCCGT- 3') generated OPB-11_{861}, a polymorphic DNA fragment 8.9 +/- 4.6 map units from Rps_4 (Table 2). The Rps_4 resistance allele was in coupling with the presence of the DNA fragment $OPB11_{861}$. We also found linkage between $OPB11_{861}$ and the RFLP markers pT-5 and pA-586 which were previously found to be linked to Rps_4 (Diers et al., 1992). pA-586 is a codominant marker, while pT-5 and $OPB11_{861}$ showed a pattern of dominant inheritance.

Multipoint linkage analyses were done using the computer program Mapmaker (Lander et al., 1987) to determine the map orientation of the markers and Rps_4 gene (Fig. 1). The map orientations obtained from these analyses were consistent with Diers et al. (1992). The addition of $OPB11_{861}$ to the linkage group has, however, resulted in a greater map distance between pA-586 and pT-5 than found by Diers et al, (1992). Diers et al. (1992) estimated the map distance between pA-586 and pT-5 to be 10 cM using a multipoint linkage test. The addition of $OPB11_{861}$ between pA-586 and pT-5 increased this distance to 15.7 cM. The changes in distance estimates are not unexpected because of the relatively small population used to map Rps_4.

The practical utility of $OPB11_{861}$ is uncertain because the Rps_4 gene is present in few soybean cultivars that would be used as parents in a breeding program. In some cases, $OPB11_{861}$ could be useful as an indirect test for Rps_4. The 8.9 map units between $OPB11_{861}$ and Rps_4 will, however, result in some false readings because of recombination.

Table 1. Pedigrees of near isogenic lines used in the study

Gene	Parentage
Rps_1	Wm79 x L76-2013
Rps_2	Wm(6)[a] x (Harosoy (5) x D54-2437)
Rps_3	Wm(6) x PI 86972-1
Rps_4	Wm(6) x PI 86050
Rps_5	Wm(6) x PI 91160
Rps_6	Wm(5) x Altona

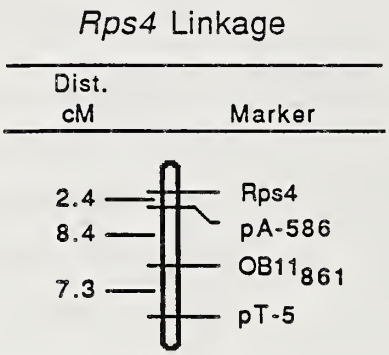
[a]Number of backcrosses used in developing the NILs

Table 2. Summary of genetic linkages in population segregating for Rps_4[a]

Linked loci	Chi-square	Pr[b]	r[c]	SE[d]
Rps_4/pT-5	19.4	.00001	12.6	5.7
Rps_4/pA-586	38.4	.00000	2.3	2.3
Rps_4/$OPB11_{861}$	27.2	.00000	8.9	4.6
pT-5/pA-586	23.7	.00000	9.8	4.9
pT-5/$OPB11_{861}$	26.8	.00000	7.4	4.3
pA-586/$OPB11_{861}$	30.3	.00000	8.7	4.5

[a]Linkage analyses were done using the computer program Linkage-1 (Suiter, et al, 1983).
[b]Probability of a greater value of chi-square by chance alone.
[c]Estimate of frequency of recombination.
[d]Standard error of recombination estimate.

Figure 1. Linkage map of Rps_4 and linked markers. The map was made using multipoint linkage analyses with the computer program Mapmaker (Lander et al., 1987).



J.R. Byrum
P.M. Kimbirauskas
R.C. Shoemaker
B.W. Diers

Acknowledgements: The authors wish to thank Dr. R.L. Bernard, University of Illinois and USDA-ARS, for developing the near isogeneic lines surveyed in this study. The authors also thank Rick Ruff, at Iowa State University, for conducting the Rps_4 phytophthora evaluation.

References:

Athow, K.L. 1987. Fungal diseases. p. 689-727. In J.R. Wilcox (ed.) Soybeans: Improvement, production, and uses. 2nd ed. Agron. Monogr. 16 ASA, CSSA, and SSSA, Madison, WI.

Athow, K.L., F.A. Laviolette, E.H. Mueller, J.R. Wilcox. 1980. A new major gene for resistance to Phytophthora megasperma var. sojae in soybean. Phytopathology 70:977-980.

Diers, B.W., L. Mansur, J. Imsande, R.C. Shoemaker. 1992. Mapping phytophthora resistance loci in soybean with restriction fragment length polymorphism markers. Crop Sci. 32:377-383.

Kaufmann, M.J., J.W. Gerdemann. 1958. Root and stem rot of soybean caused by Phytophthora sojae n. sp. Phytopathology 48:201-208.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, L. Newburg. 1987. Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Martin, G., J. Williams, S.D. Tanksley. 1991. Rapid identification of markers linked to a Psuedomonas resistance gene in tomato by using random primers and near-isogenic lines. Proc. Natl. Acad. Sci. 88:2336-2340.

Michelmore, R.W., I. Paran, R.V. Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions using segregating populations. Proc. Natl. Acad. Sci. 88:9828-9832.

Miklas, P.N., J.R. Stavely, J.D. Kelly. 1993. Identification and potential use of a molecular marker for rust resistance in common bean. Theor. Appl. Genet. 85:745-749.

Morgan, F.L., E.E. Hartwig. 1965. Physiological specialization in Phytophthora megasperma var. sojae. Phytopathology 55:1277-1279.

Paran, I., R.V. Kesseli, R.W. Michelmore. 1991. Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. Genome 34:1021-1027.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen, R.W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. 81:8014-8018.

Sarfatti, M., J. Katan, R. Fluhr, D. Zamir. 1989. An RFLP marker in tomato linkeld to the Fusarium oxysporum resistance gene I2. Theor. Appl. Genet.78:755-759.

Suiter, K.A., J.F. Wendel, J.S. Case. 1983. Linkage-1: A Pascal computer program for the detection and analysis of genetic linkage. J. Hered. 74:203-204.

Williams, J.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, S.B. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

Young, N.D., D. Zamir, M.W. Ganal, S.D. Tanksley. 1988. Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the Tm-2a gene in tomato. Genetics 120:579-585.

IOWA STATE UNIVERSITY
Ames, IA 50011

245

Genetic linkage in soybean: Linkage group 8

Introduction: Based on chromosome number, 20 linkage groups are expected in soybean. Linkage studies using conventional markers have defined 20 linkage groups (Palmer and Kiang, 1990; Muehlbauer et al., 1989; Devine et al., 1991; Palmer et al., 1992). These may not necessarily correspond with 20 different chromosomes since only two linkage groups have more than four markers. Only one linkage group has been assigned to a chromosome. Linkage Group 8 has been assigned to the satellite or nucleolus organizing chromosome (Sadanaga and Grindeland, 1984) and is one of the better described linkage groups.

Linkage Group 8 is defined by seven known gene loci: W1, Wm, Ms1, St5, Adh1, Ms6, and Y23 (Table 1). Buzzell et al. (1977) linked W1 with Wm. Palmer and Kaul (1983) linked W1 with Ms1 and with St5. Kiang and Chiang (1987) linked W1 with Adh1. Skorupska and Palmer (1989) linked W1 with Ms6. Palmer et al. (1990) linked Y23 with W1 and Ms6.

Table 1. The loci of soybean Linkage Group 8

Locus	Phenotype of recessive homozygote	Source of recessive allele
W1	White flower	Many cultivars
Wm	Magenta flower	T235[a]
Ms1	Male sterile, female fertile	T266H[a]
St5	Male sterile, female sterile	T272H[a]
Adh1	Alcohol dehydrogenase band 1 absent	'Wye' and other cultivars
Ms6	Male sterile, female fertile	T295H[a]
Y23	Chlorophyll-deficient foliage	T288[a]

[a] Genetic Type Collection number.

Palmer and Kaul (1983) proposed gene order as Ms1 - chromosome translocation breakpoint - W1 - Wm - St5, Palmer (1985) proposed gene order as Ms1 - Wm - W1 - St5, and Kiang (1990) proposed gene order as Ms1 - Wm - W1 - Adh1. Palmer et al. (1990) showed Y32 to be very close to St5. Palmer et al. (1990) also showed percentage recombination between Y23 and Ms6 to be less than between Y23 and W1. The gene order of the seven known loci needs to be determined.

The expectations of gene order are based on many different data sets of segregation of two loci each (two-point data). Linkage maps based on segregation of three or more loci would be preferred to determine order. Published linkage estimates have been calculated using the product method (Immer and Henderson, 1943), the maximum likelihood method (Allard, 1956), and the computer software program, LINKAGE-1 which is based on the maximum likelihood method (Suiter et al., 1983). Estimates have been based on both F1:2 and F2:3 data. Published data involve many different genotypes and environments (locations and seasons). Recombination values can be influenced by the genotype and selfing environment (Mock, 1972; Pfeiffer and Vogt, 1990).

The purpose of this study is to provide additional three-point linkage data for six of the loci in Linkage Group 8 (all but Wm) using a common parent to produce F1 seed, and advancing the F1 seeds to the F2 in relatively similar environments. We decided to make cross pollinations between 'BSR 101', which is of the dominant genotype at all loci being studied (Haack et al., 1992), and individuals with sets of three of the mutant alleles in coupling. The W1 locus was involved in all cross combinations. The F2 were scored for the phenotypic traits and linkage estimates were made based upon the percentage of recombination between parental alleles.

Materials and methods: Eight lines were created which were homozygous for the recessive allele at the W1 locus and two other recessive alleles in coupling phase. These lines are listed in Table 2.

Table 2. Listing and classification of soybean lines with three mutant alleles in coupling

adh1 lines	y23 lines	Double sterile lines
w1 adh1 ms1	w1 ms1 y23	w1 ms1 st5
w1 adh1 ms6	w1 ms6 y23	w1 ms6 st5
	w1 st5 y23	w1 ms1 ms6

adh1 lines:

Seeds from the w1 adh1 ms1 line and the w1 adh1 ms6 line were sown in the field at the Bruner farm near Ames, Iowa in 1990. Remnant seed from each family was scored for adh1 using the method described by Haack et al. (1992) to find families for crossing that were adh1 adh1. White flowered, adh1 adh1 ms1 ms1 and adh1 adh1 ms6 ms6 plants were used as pod parents in crosses with BSR 101. The F1 seeds were advanced to the F2 at the University of Puerto Rico / Iowa State University Soybean Nursery, Isabela, Puerto Rico. F2 seeds were tested for adh1 and seedlings were transplanted to peat pots and then to the field. Seedling identity was maintained. The plants were scored at flowering for flower color and at maturity for fertility/sterility.

y23 lines:

White-flowered, chlorophyll-deficient sterile plants in the w1 ms1 y23 and w1 ms6 y23 lines were used as pod parents in crosses with BSR 101. White-flowered, chlorophyll-deficient fertile plants in the w1 st5 y23 line were used as pollen parents in crosses with BSR 101 and given an identification number for use in progeny testing to determine the genotype of each parent, St5 St5 or St5 st5. The F1 seeds were advanced to the F2 in Puerto Rico. Selfed seeds from w1 w1 y23 y23 St5 -- plants used as parents were grown in a progeny test in Puerto Rico and scored for segregation of fertility/sterility. A segregating family identifies a heterozygous parent; only F2 seed from heterozygous parents were used in the linkage study. F2 plants were scored at flowering for flower color and plant color. At maturity, plants were scored fertile or sterile. Only one family, A91-157, from crosses with w1 w1 y23 y23 St5 -- plants segregated for all three traits. Green, fertile, purple-flowered plants from this family were harvested, and F2:3 progeny

were evaluated to obtain F3 data. Also, to generate additional linkage data, two plants in A91-157 that were w1 w1 y23 y23 St5 -- were used as pollen parents with BSR 101. The F1 were advanced to the F2 in Puerto Rico. Seven F1:2 families segregated for all three traits, and individual plants were classified the following season.

Double sterile lines:

Individual ms1 ms1 plants in the w1 ms1 st5 line and ms6 ms6 plants in the w1 ms6 st5 line were used as pod parents in crosses with BSR 101. We believed that many of these ms6 ms6 plants would be St5 st5 and would transmit the st5 allele half of the time. Individual ms6 plants in the w1 ms1 ms6 lines were used as pod parents in crosses with BSR 101. We believed that many of these ms6 ms6 plants would be ms1 ms1. We also knew the ms6 phenotype was epistatic over the ms1 phenotype. The F1 seed were advanced to the F2 in Puerto Rico. F2 plants from crosses with the w1 ms1 st5 line were scored at flowering for flower color. Flower buds were taken at this time to score for fertility and type of sterility; these two sterile mutants cannot be distinguished without the aid of a light microscope. Pollen was scored fertile, ms1 ms1, st5 st5, or ms1 ms1 st5 st5 double sterile. Pollen grains of the ms1 ms1 st5 st5 double sterile plants were not uniform in grain size or degree of staining. Size and degree of staining ranged from as large and dark as pollen from ms1 ms1 plants (Albertsen and Palmer, 1979) to as small and light as pollen from st5 st5 plants (Palmer and Kaul, 1983). The majority of pollen was medium staining and sized between normal pollen and pollen from ms1 ms1 plants. The centers usually were shrunken away from the edges like pollen from st5 st5 plants (Palmer and Kaul, 1983). F2 plants from crosses with the w1 ms6 st5 line were scored at flowering for flower color and fertility or type of sterility. Plants scored ms6 ms6 were scored again at maturity for pod-set/no pod-set. Individuals without pod set were classified as ms6 ms6 st5 st5 double steriles because of the near-complete sterility of the st5 sterile (Palmer and Kaul, 1983), while individuals with pod set were classified as single steriles because of the tendency of the ms6 male-sterile to set several outcross pods (Skorupska and Palmer, 1989). F2 plants from crosses made with the w1 ms1 ms6 line were scored at flowering for flower color and fertility or type of sterility. Double ms1 ms1 ms6 ms6 steriles could not be distinguished from ms6 ms6 single steriles due to epistasis

of the ms6 phenotype over the ms1 phenotype at both flowering and maturity. Fertile purple-flowered plants were harvested, and the F2:3 families were classified to obtain F3 data.

Chi-square values and percentage recombination values for F1:2 data were calculated using the computer software program LINKAGE-1 (Suiter et al., 1983). Chi-square calculations for F2:3 data were made using the formula: Chi-square = Sum [|no. observed - no. expected| - 0.5)^2]/no. expected. Percentage recombination values for F2:3 data were calculated using a modification of the product method (Allard, 1956), or by the method described by Hanson (1959) for data with a missing recombination class. The data from any family were used only if the data for all loci in that family fit the expected 3:1 ratio (5% probability level).

Results and discussion: The percentage recombination values of this study generally agree with published values. Some of our percentage recombination values are very close to published values, some are slightly different than published values, and some are considerably different than published values. A summary of recombination percentages from F1:2 data is presented in Table 3. A summary of recombination percentages from F2:3 data is presented in Table 4.

The percentage recombination values of this study indicate a gene order that agrees with the expected gene order. Our data give gene order as Ms1 - W1 - Ms6 - Y23 - St5 - Adh1 or as Ms1 - W1 - Ms6 - St5 - Y23 - Adh1. Due to overlapping standard errors, our data do not distinguish the order of St5, Y23, and Adh1 relative to W1 and Ms6. Two alternative Linkage Group 8 maps are given (Figures 1 and 2). One map is based on percentage recombination values between the W1 locus and the other loci. The other map is based on percentage recombination values between the Ms6 locus and the other loci.

To determine gene order of loci Adh1, Y23 and St5 in relation to W1, we have decided to generate F1:2 data involving these four loci in coupling. Cross pollinations have been made to create a w1 w1 adh1 adh1 y23 y23 St5 st5 line. Fertile plants, St5 St5 or St5 st5, from this line will be crossed with BSR 101. The F2 from crosses involving heterozygous

parents (St5 st5) will be scored for the four traits involved, and percentage recombination values will be calculated.

References:

Albertsen, M.C., and R.G. Palmer. 1979. A comparative light- and electron-microscopic study of microsporogenesis in male sterile (ms1) and male fertile soybeans [Glycine max (L.) Merr.]. Amer. J. Bot. 66:253-265.

Allard, R.W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia. 24:235-278.

Buzzell, R.I., B.R. Buttery, and R.L. Bernard. 1977. Inheritance and linkage of a magenta flower gene in soybeans. Can. J. Genet. Cytol.19:749-751.

Devine, T.E., T.C. Kilen, and J.J. O'Neill. 1991. Genetic linkage of the phytophthora resistance gene Rps2 and the nodulation response gene Rj2 in soybean. Crop Sci. 31:713-715.

Haack, K.S., T.D. Couch, J. Imsande, and R.G. Palmer. 1992. A starch gel electrophoretic procedure for resolving soybean alcohol dehydrogenase band 1. Soybean Genet. Newsl. 19:92-97.

Hanson, W.D. 1959. Minimum family sizes for the planning of genetic experiments. Agron. J. 51:711-715.

Immer, F.R., and M.T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.

Kiang, Y.T. 1990. Mapping the alcohol dehydrogenase locus (Adh1) in soybean Linkage Group 8. J. Hered. 81:488-489.

Kiang, Y.T., and Y.C. Chiang. 1987. Genetic linkage of the Adh1 and W1 loci in soybeans. Genome 29:582-583.

Mock, J.J. 1972. Manipulation of crossing over with intrinsic and extrinsic factors. Egypt. J. Genet. Cytol. 2:158-175.

Muehlbauer, G.J., J.E. Specht, P.E. Staswick, G.L. Graef, and M.A. Thomas-Compton. 1989. Application of the near-isogenic line gene mapping technique to isozyme markers. Crop Sci. 29:1548-1553.

Palmer, R.G. 1985. Soybean cytogenetics. p. 337-344. *In* R. Shibles (ed.) Proc. World Soybean Res. Conf. III., Westview Press, Boulder, Colorado.

Palmer, R.G., and M.L.H. Kaul. 1983. Genetics, cytology, and linkage studies of a desynaptic soybean mutant. J. Hered. 74:260-264.

Palmer, R.G., and Y.T. Kiang. 1990. Linkage map of soybean [Glycine max (L.) Merr.]. p. 6.68-6.93 *In* S.J. O'Brien (ed.) Genetic Maps. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Palmer, R.G., S.M. Lim, and B.R. Hedges. 1992. Testing for linkage between the Rxp locus and nine isozyme loci in soybean. Crop Sci. 32:681-683.

Palmer, R.G., R.L. Nelson, R.L. Bernard, and D.M. Stelly. 1990. Genetics and linkage of three chlorophyll-deficient mutants in soybean: y19, y22, and y23. J. Hered. 81:404-406.

Pfeiffer, T.W., and S.D. Vogt. 1990. Variability for recombination frequencies in the AP12 soybean population. Crop Sci. 30:545-549.

Sadanaga, K., and R.L. Grindeland. 1984. Locating the w1 locus on the satellite chromosome in soybean. Crop Sci. 24:147-151.

Skorupska, H., and R.G. Palmer. 1989. Genetics and cytology of the ms6 male-sterile soybean. J. Hered. 80:304-310.

Suiter, K.A., J.F. Wendel, and J.S. Case. 1983. LINKAGE-1: a PASCAL computer program for the detection and analysis of genetic linkage. J. Hered. 74:203-204.

Kim S. Lewers
Reid G. Palmer USDA-ARS

Table 3. Summary of percentage recombination values, by loci pair, across all F1:2 experiments

Loci pair	Experiment	No. F1 plants	No. F2 plants	R ± SE[a]
W1 - Adh1	W1 Adh1 Ms1	3	300	17.95 ± 3.57
	W1 Adh1 Ms6	8	665	20.52 ± 2.63
	Sum	**11**	**965**	**19.59 ± 2.12**
W1 - Ms1	W1 Adh1 Ms1	3	300	33.04 ± 5.56
	W1 Ms1 Y23	5	683	28.04 ± 3.05
	W1 Ms1 St5	2	649	30.72 ± 3.37
	Sum	**10**	**1632**	**29.97 ± 2.10**
Adh1 - Ms1	W1 Adh1 Ms1	3	300	41.25 ± 6.56
W1 - Ms6	W1 Adh1 Ms6	8	665	3.96 ± 0.99
	W1 Ms6 Y23	4	1167	3.86 ± 0.69
	W1 Ms6 St5	3	329	4.78 ± 1.46
	Sum	**15**	**2161**	**4.04 ± 0.53**
Adh1 - Ms6	W1 Adh1 Ms6	8	665	16.48 ± 2.27
W1 - Y23	W1 St5 Y23[b]	7	645	18.81 ± 2.38
	W1 St5 Y23	1	251	16.69 ± 6.10
	W1 Ms1 Y23	5	683	19.07 ± 2.31
	W1 Ms6 Y23	4	1167	16.89 ± 1.63
	Sum	**17**	**2745**	**17.73 ± 1.05**
Ms1 - Y23	W1 Ms1 Y23	5	683	38.80 ± 3.90
Ms6 - Y23	W1 Ms6 Y23	4	1167	13.30 ± 1.40
W1 - St5	W1 St5 Y23[b]	7	645	19.36 ± 2.43
	W1 St5 Y23	1	251	18.53 ± 6.05
	W1 Ms1 St5	2	649	18.94 ± 2.37
	W1 Ms6 St5	3	329	16.36 ± 3.01
	Sum	**13**	**1874**	**18.56 ± 1.29**
St5 - Y23	W1 St5 Y23[b]	7	645	0.96 ± 0.45
	W1 St5 Y23	1	251	2.37 ± 1.15
	Sum	**8**	**896**	**1.37 ± 0.46**
Ms1 - St5	W1 Ms1 St5	2	649	38.57 ± 4.00
Ms6 - St5	W1 Ms6 St5	3	329	11.05 ± 2.35

[a]R ± SE is percentage recombination plus or minus standard error.
[b]Data from the second season, summer 1992.

Table 4. Summary of percentage recombination values, by loci pair, from F2:3 experiments

Loci pair	Experiment	No. F1 families	No. F2 families	R ± SE[a]
W1 - Ms1	W1 Ms1 Ms6	5	152	33.62 ± 5.84
W1 - Ms6				2.49 ± 0.77
Ms1 - Ms6				37.86 ± 6.68
W1 - Y23	W1 St5 Y23	1	147	16.96 ± 2.72
W1 - St5				17.70 ± 2.83
St5 - Y23				≤1.01[b]

[a]R ± SE is percentage recombination plus or minus standard error.
[b]Estimate of the maximum recombination value calculated using the method described by Hanson (1959).

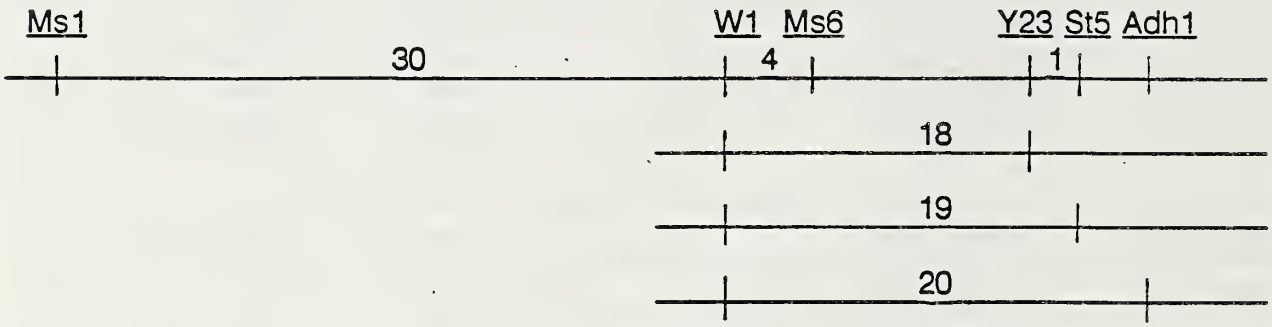


Figure 1. Linkage map based on summary percentage recombination values between the W1 locus and the other loci

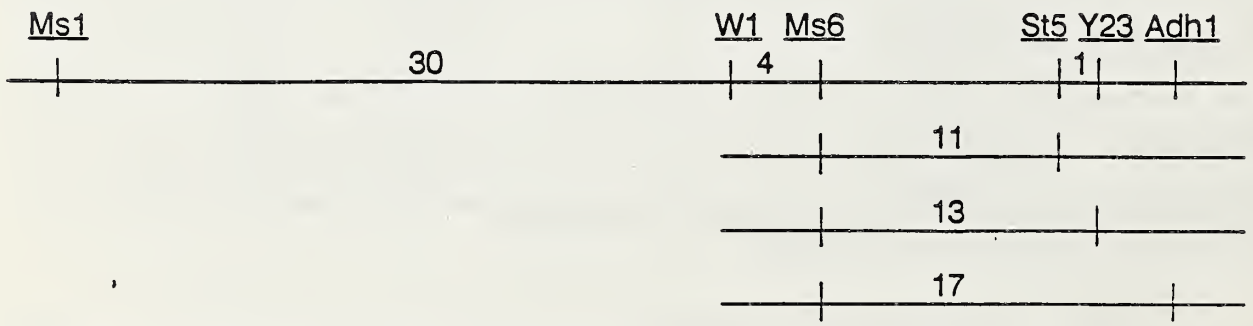


Figure 2. Linkage map based on summary percentage recombination values between the Ms6 locus and the other loci.

Iowa State University
Departments of Agronomy and Zoology and Genetics
USDA-ARS
Ames, Ia 50011

245

Molecular relatedness among ancestral 'types'

Between the years 1910 and 1913, the old domestic soybean varieties, AK, Manchu, Manchuria, and Mandarin were introduced into the United States from China. These varieties were distributed throughout the United States and Canada, and in the following 38 years, variants were detected, selected, and released as 'new' varieties based on visible phenotypic differences (Bernard, et al., Technical Bulletin, 1988). The selected and released variants are sometimes referred to as 'types'; for example, AK(Harrow) is an AK type. This implies a similarity to AK, however, the extent of actual genetic similarities among 'types' is unknown.

Four AK 'types', seven Manchu 'types', three Manchuria 'types' and three Mandarin 'types' were for RFLP analysis , to determine the amount of similarity these 'types' have to their progenitor, and to each other (Table 1).

Thirty-one RFLP probes, representing 32 mapped loci, were used against these 17 varieties. These probes were chosen based on their probability of detecting polymorphisms among other soybean varieties (Lorenzen, unpublished data), with the intent to minimize the number of uninformative probes used. Only the probe/enzyme combinations used in creating the USDA:RFLP molecular map were used, and only those restriction fragment polymorphisms identical to those used in mapping are reported (Diers, et al., 1992).

Table 1. Eighteen old domestic soybean varieties, representing four 'types' are shown along with their year of selection, and their country or state of origin

Variety	Year selected	Country/State of Origin	Comments
AK	1912	China	Seed Not Available
AK(FC30.761)	1940	Kentucky	
AK(Harrow)	1928	Canada	Reported as "identical to Illini"
AK(Kansas)	1949	Kansas	
Illini	1920	Illinois	
Manchu	1911	China	
Manchu 3	1940	Wisconsin	
Manchu 606	1940	Wisconsin	
Manchu 2204	1942	Ohio	
Manchu (Lafayette)	1943	Indiana	
Manchu (Madison)	1951	Wisconsin	
Manchu (Montreal)	1944	Canada	
Manchuria	1910	China	
Manchuria 13177	1913	Ohio	
Manchuria 20173	1920	Ohio	
Mandarin	1913	China	
Mandarin 507	1943	Wisconsin	
Mandarin (Ottawa)	1929	Canada	

A matrix of the proportion of polymorphic loci between the AK 'types' can be seen in Table 2. AK(Harrow) and Illini (reported as 'identical') are the most similar, with seven out of 32 probes detecting differences (0.22), while AK(Kansas) differs by a proportion of 0.47 with both AK(FC30.761) and AK(Harrow).

Table 2. Proportion of polymorphic loci detected among the AK 'types', based on 32 loci.

	AK(FC30.761)	AK(Harrow)	AK(Kansas)
AK(Harrow)	0.31		
AK(Kansas)	0.47	0.47	
Illini	0.44	0.22	0.44

Among the Manchu 'types', the differences range from no polymorphisms detected between Manchu 3 and Manchu 606, to a proportion of 0.41 polymorphic loci detected (13 out of 32 loci) between Manchu (Lafayette) and four other Manchu 'types' (Table 3).

Table 3. Proportion of polymorphic loci detected among the Manchu 'types', based on 32 loci.

	Manchu	Manchu 3	Manchu 606	Manchu 2204	Manchu (Lafayette)	Manchu (Madison)
Manchu 3	0.19					
Manchu 606	0.19	0.00				
Manchu 2204	0.13	0.16	0.16			
Manchu (Lafayette)	0.41	0.41	0.41	0.41		
Manchu (Madison)	0.31	0.38	0.38	0.38	0.16	
Manchu (Montreal)	0.34	0.28	0.28	0.28	0.38	0.28

Table 4 shows the proportion of polymorphic loci for the Manchuria 'types'. Manchuria 13177 and Manchuria 20173, both selected in Ohio, show the lowest proportion of polymorphic loci (0.13, four out of 32 loci), while Manchuria and Manchuria 13177 appear to be very polymorphic (0.50, 16 out of 32 loci).

Table 4. Proportion of polymorphic loci detected among the Manchuria 'types', based on 32 loci.

	Manchuria	Manchuria 13177
Manchuria 13177	0.50	
Manchuria 20173	0.44	0.13

The Mandarin 'types' are shown in Table 5. Mandarin 507 and Mandarin (Ottawa) are the most similar, with only five out of 32 loci (0.16) exhibiting differences, while Mandarin and Mandarin 507 are the most different (0.31, 10 out of 32 loci polymorphic).

Table 5. Proportion of polymorphic loci detected among the Mandarin 'types', based on 32 loci.

	Mandarin	Mandarin 507
Mandarin 507	0.31	0.00
Mandarin (Ottawa)	0.25	0.16

It is evident from this analysis that, with only one exception (Manchu 3 and Manchu 606), the varieties within a given 'type' can be distinguished from each other at the molecular level, and often appear to be very different from their progenitor and each other. Thus, it is important when reporting pedigrees to include the full name, (i.e. Manchu 3) and not just the 'type' (i.e. Manchu). Possible causes for these differences include the original selection being heterogeneous, 'foreign' seed being inadvertantly mixed in with the progenitor seed, or mislabeling a seed source. These variants could also be the result of an outcross. Additional work is being done to further characterize these and other ancestoral lines.

References

Bernard, R.L., G.A. Juvik, E.E. Hartwig, and C.J. Edwards, Jr. 1988. Origins and Pedigrees of Public Soybean Varieties in the United States and Canada. Technical Bulletin Number 1746. National Technical Information Service, Springfield, VA.

Diers, B.W., P. Kiem, W.R. Fehr, and R.C. Shoemaker. 1992. RFLP analysis of soybean seed protein and oil content. Theor. Appl. Genet. 83:600-612.

Lisa L. Lorenzen
Kimberly J. Reth
Randy C. Shoemaker, USDA-ARS

PLANT SCIENCES INSTITUTE
Plant Molecular Biology Laboratory
USDA, ARS
Beltsville, Maryland 20705

Tests for genetic linkage of the *Rj4* gene and other genes

The Rj4 allele in soybean, [*Glycine max* (L.) Merr.] conditions an ineffective nodulation response primarily with bradyrhizobial strains. These stains are classified as *Bradyrhizobium elkanii* (Kuykendall et al., 1992), which are less efficient in N2 fixation with soybean, thus protecting the plant from many of these strains. The Rj4 phenotype is the most common phenotype (63%) in the USDA Soybean Germplasm Collection of *Glycine soja* Sieb. & Succ., the wild undomesticated progenitor of the cultivated soybean (Devine, 1987). It is also the most common phenotype in the plant introductions of the domesticated soybean from Southeast Asia (Devine and Breithaupt, 1981). However, the frequency of the Rj4 allele has diminished with selection for agronomic type in North America (Devine and Breithaupt, 1981). Whether this is due to random drift, a negative selection value for the Rj4 allele itself, or to linkage of the Rj4 allele with other genes with undesirable agronomic traits is unresolved (Devine, 1987).

The Rj4 gene has been determined to segregate independently of loci in nine of the linkage groups in the classical genetic map of soybean. Devine (1992) reported the Rj4 gene to be independent of the Y12 gene in Linkage Group 1, the P gene in Linkage Group 2, the Ln gene in Linkage Group 4, the L1 gene in Linkage Group 5, the I gene in Linkage Group 6, the W1 gene in Linkage Group 8, the F gene in Linkage Group 11, the Fr1 gene in Linkage Group 12, the Y9 gene in Linkage Group 14, and the as yet unmapped genes Fr2, Y10, and Y17.

F2 plants from the cross 'Peking' (Rj4, i, T) x 'Hardee' (rj4, i-i, t) were evaluated in the greenhouse and field at Beltsville, MD in order to study the segregation of the Rj4 locus with the I and T loci. The plants were first planted

in the greenhouse in sterile vermiculite and inoculated with a broth culture of strain USDA 61 from the Beltsville Rhizobium Culture Collection. Strain USDA 61 distinguishes the Rj4 vs. rj4 phenotypes. After two weeks, the roots were examined and classified for nodulation response. Plants were then transplanted to the field and grown to maturity, at which time the pubescence color (T) and seed coat color (I) were recorded.

F2 plants from the cross T288 (y23, rj4) x BARC-2(Rj4) (y23, Rj4) were planted in the greenhouse in Beltsville, MD to study the segregation of the Rj4 locus with the Y23 locus. These plants were also planted in sterile vermiculite and inoculated with strain USDA 61. The chlorotic seedlings were marked after 2 wk with buff colored correction fluid. After 4 wk, the plants were scored for nodulation restriction by the Rj4 allele.

Gene symbols given in Table 1 refer to genetic traits described by Palmer and Kilen (1987). Recombination was calculated from F2 data using the method of maximum likelihood, as described by Allard (1956) and Mather (1951), except where monogenic ratios deviated significantly from the expected, in which case the product moment method (Immer and Henderson, 1943) was used to calculate recombination frequency. The bisection method was used to solve the maximum likelihood equations (Yakowitz and Szidarovszky, 1989).

Chi-square analysis indicated that the monogenic ratios gave a good fit to a 3:1 ratio except for the Rj4 gene in both crosses. Therefore, the product moment method was used to estimate recombination in tests with the Rj4 gene. The linkage chi-squares indicated independent assortment of the Rj4 locus with the T, I, and Y23 loci and of the T locus with the I locus. In this study, we have determined that Rj4 is not linked to the T locus and in a previous study we determined that Rj4 is not linked to the Y12 locus (Devine, 1992). The T locus and the Y12 locus are linked together in classical Linkage Group 1 (Palmer and Kilen, 1987). Also in this study we have determined that Y23 in Linkage Group 8 is not linked to the Rj4 locus. In a previous study, we determined that Rj4 was not linked to W1. W1 and Y23 are linked together with 28% recombination in Linkage Group 8 (Palmer and Kilen, 1987).

References:

Allard, R. W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235-278.

Devine, T. E. 1987. A comparison of rhizobial strain compatibilities of *Glycine max* and its progenitor species *Glycine soja*. Crop Sci. 27:635-639.

Devine, T. E. 1992. Genetic linkage tests for the Rj4 gene in soybean. Crop Sci. 32:961-964.

Devine, T. E., B. H. Breithaupt. 1981. Frequencies of nodulation response alleles, Rj2 and Rj4, in soybean plant introduction breeding lines. USDA Tech. Bull. 1628. U.S. Gov. Print. Office, Washington, DC.

Immer, F. R., M. T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.

Kuykendall, L. D., B. Saxena, T. E. Devine, S. E. Udell. 1992. Genetic diversity in *Bradyrhizobium japonicum* Jordan 1982 and a proposal for *Bradyrhizobium elkanii* sp. nov. Can. J. Microbiol. 38:501-505.

Mather, K. 1951. The measurement of linkage in heredity. 2nd ed. Methuen & Co., London.

Palmer, R. G., T. C. Kilen. 1987. Qualitative genetics and cytogenetics, p. 135-209. *In* J. R. Wilcox (ed.) Soybeans: Improvement, production, and uses. 2nd ed. Agron. Monogr. 16:ASA, CSSA, and SSSA, Madison, WI.

Yakowitz, S., F. Szidarovszky. 1989. An introduction to numerical computations. 2nd ed. Macmillan Publ., New York.

T. E. Devine

Table 1. Results of genetic linkage tests of the *Rj4* gene with other loci.

Cross	Genes	Genotype† a	b	c	d	N	X2L*	P(X2L)§	Rec¶	SE#	Phase††
Peking x Hardee	T, i	82	26	26	4	138	1.1626	0.2-0.3	41	7	R
(*i, Rj4, T*) x (*i-i, rj4, t*)	T, Rj4	77	34	17	13	141	1.3246	0.2-0.3	44	6	C
	i, Rj4	73	35	19	11	138	0.0806	0.90-0.95	52	6	R
T288 x BARC-2(*Rj4*) (*rj4, y23*) x (*Rj4, Y23*)	y23, Rj4	92	20	42	6	160	1.1111	0.2-0.3	55	6	C

† Class designations per Allard, 1956.

* Linkage chi-square, partitioned from the total chi-square, was calculated with a 9:3:3:1 ratio expected in the F_2, with 1 df for linkage.

§ Chi-square probability

¶ Rec = estimate of the recombination frequency using the product method for Rj4 where single factor ratios were disturbed, or the method of maximum likelihood for the T vs I test

\# SE = standard error or recombination estimate

†† Phase: C = Coupling, R = Repulsion

UNIVERSITY OF MINNESOTA
(1) Department of Plant Pathology
(2) Department of Agronomy and Plant Genetics
St. Paul, Minnesota 55108
UNIVERSITY OF THE PHILIPPINES at LOS BANOS
(3) Institute of Plant Breeding
College, Laguna 4031 Philippines

245

RFLP Mapping of Cyst Nematode Resistance Genes in Soybeans

Summary: The soybean cyst nematode (SCN), Heterodera glycines (Ichinohe), is one of the most destructive pests of soybean in Minnesota and throughout the midwest. To date, host plant resistance has been the most cost-effective control measure. However, resistance to SCN is polygenic and complex, screening germplasm is tedious and SCN race determination is difficult. Thus, conventional breeding for SCN resistance is time-consuming and extremely difficult. A more efficient approach is the use of DNA genetic markers, such as restriction fragment length polymorphisms (RFLPs), to identify loci tightly linked to SCN resistance genes. To uncover these associations, a segregating F2 population was constructed by crossing a susceptible (M83-15) and a resistant (M85-1430) soybean inbred line. Using RFLPs and random amplified polymorphic DNAs (RAPDs), fifty-six F2 lines were mapped for marker polymorphism. At the same time, F3 lines derived from these F2 lines were then assayed for SCN disease response against a field isolate of SCN from Minnesota that behaved as race 3. The F2 genotypic classes for each DNA marker were then contrasted with SCN disease response to identify loci associated with SCN resistance. Two genomic regions were found to be significantly associated with SCN disease response, together accounting for 56% of the total phenotypic variation. Based on RFLP analysis, individual F2 lines that have retained either one or both of the resistance loci were identified. Individual lines that possess both of the resistance loci can be valuable in developing resistant soybean lines free of linkage drag, while lines that have

retained either one of the resistance loci can be potentially used as single-gene differentials for SCN race determination.

Materials and methods: The segregating F2 population was constructed by crossing two contrasting soybean lines, M85-1430 with a PI 209332 resistance, and a susceptible line M83-15, and advancing it to the F2 generation. Fifty-six F2 individuals, together with the parents, were grown in a greenhouse in St. Paul, Minnesota, and used as source of leaf tissue for DNA extraction and RFLP analysis. Plants were allowed to recover and set F3 seeds, which were saved for SCN disease assay. DNA extraction, restriction digests, electrophoresis, Southern blots, hybridization, and autoradiography and the polymerase chain reaction technique for RAPDs were done following conventional methods (Dellaporte, 1983; Southern, 1975; Young et al., 1992). A total of 25 F3 seedlings for each F2 line were assayed for SCN resistance using both the waterbath and bench method. Each plant was inoculated with 1,000 SCN eggs of a field isolate from Minnesota that behaved as race 3. Inoculations were done twice; once at transplanting and again two days later. Soil temperatures were maintained at 28°C at 16-hour daylength for 28 days. On the 28th day, individual plants were uprooted and cysts were collected by blasting the roots with pressurized water. The total number of cysts from individual plants was counted under a dissecting microscope and converted to an index by dividing this number by the total number of cysts on the susceptible parent. Regression analysis and analysis of variances were used to uncover associations between the DNA markers and SCN disease response. Because of the large number of DNA markers tested for association with SCN resistance, a level of significance of $p<.002$ (Lander and Botstein, 1989) was chosen to ensure no false positives experiment-wide.

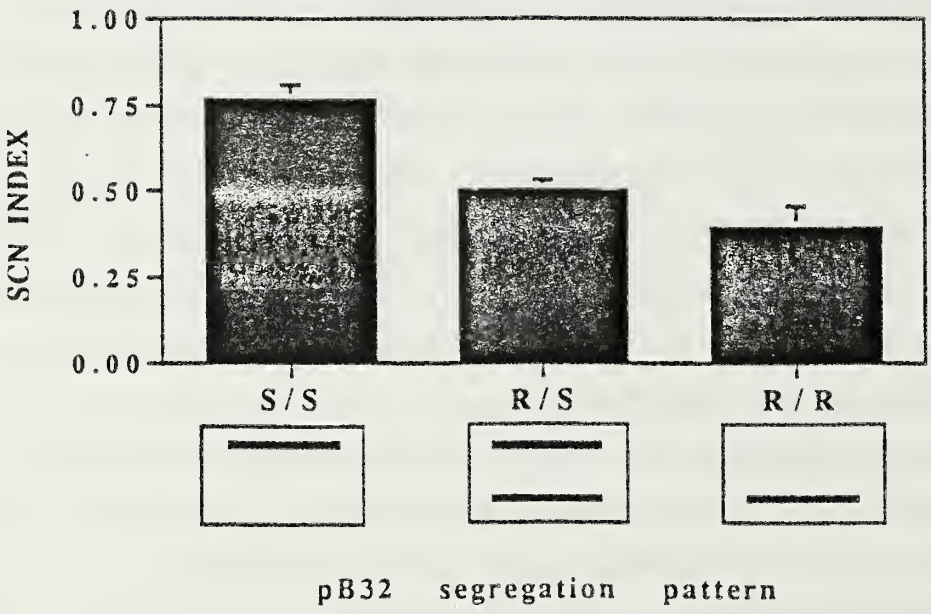
Results and discussion: Of the 45 DNA markers that were analyzed, two RFLP markers, pB32 and pA85, were found to be strongly associated with SCN disease response controlling 39% and 23% of the total variation in SCN disease response, respectively (Table 1). Figure 1 shows that for RFLP marker pB32, individual plants that are homozygous for M85-1430 allele (resistant parent), are

48% more resistant on average than homozygous individuals for M83-15 allele (susceptible parent). Based on a two-factor analysis of variance, together these two markers accounted for 56% of the total variation. One of the markers on linkage group A, pA85, also showed strong association with hilum color, a seed character previously reported to be associated with SCN disease response (Matson and Williams, 1965). On the other hand, the genomic region where pB32 was located was defined by three other RFLP markers, one of which is pK417, which we previously reported to be a major locus controlling SCN resistance (Concibido et al., 1992).

Table 1. Individual major locus-effects on SCN disease response.

Marker-Loci	F-value	P-value	R-squared
pB 32	18.034	0.0001	0.39
pA 85	7.657	0.0012	0.23

Figure 1. Individual effect of RFLP marker pB 32 on SCN disease response.



Now that the locations of putative SCN resistance loci have been determined, F2 lines that retained all the resistance loci in combination with desirable agronomic characters can be identified as potential materials for developing resistant soybean varieties. We are also currently examining the individual effects of each putative resistance locus without the influence of other resistance loci.

Briefly, lines that are fixed for all putative resistance loci except one that segregates as a Mendelian factor, were selected and challenged with an isolate of SCN. Using these RFLP markers, the ability of each putative resistance locus to distinguish SCN races can also be determined. Single gene differentials can then be developed from individual lines that are fixed for either one of the resistance loci. Finally, we can potentially combine different sources of resistance into a common soybean background by pyramiding genes.

References:

Concibido, V., R. Denny, S. Boutin, R. Hautea, S. Ansari, J, Orf, N. Young. 1992. RFLP mapping of cyst nematode resistance genes in soybean (Glycine max). Proceedings of the 4th Biennial Conference on the Molecular and Cellular Biology of Soybean. Iowa State University, Ames, Iowa 50011.

Dellaporte, S. L., J. Wood, J.B. Hicks. 19830.) A plant DNA minipreparation: version II. Pl. Mol. Biol. Rep. 1:19-21.

Lander, E. S., D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185.

Matson, A. L., L. F. Williams. 1965. Evidence of a fourth gene for resistance to soybean cyst nematode. Crop Sci 5:477.

Southern, E. 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.

Young, N. D., L. Kumar, D. Menancio-Hautea, D. Danesh, N.S. Talekar, S. Shanmugasundarum, D. H. Kim. 1992 RFLP mapping of a major bruchid resistance gene in mungbean (Vigna radiata, L. Wilczek).

V. Concibido (1)
R. Denny (1)
S. Boutin (1)
R. Hautea (3)
J. Orf (2)
N. Young (1)

UNIVERSITY OF MISSOURI
Delta Center
Portageville, MO 63873

24T

Anatomy of tolerance to soybean cyst nematode

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is by far the most serious pest of soybeans in the United States. The losses due to SCN are estimated to be over $300 million a year. Traditionally, the disease is controlled by the use of race specific resistant varieties. However, because of the presence of a large number of races of SCN, these varieties succumb to the pest. Boerma and Hussey (1984) reported PI 97,100 to have a high degree of tolerance to SCN. The tolerance to SCN was measured by comparing the seed yield from nematicide treated vs. non-treated plots. Anand and Koenning (1986) observed PI 97,100 heavily infected with both SCN Races 3 & 4, yet, exhibiting high tolerance to SCN when compared to 'Essex'.

Histological responses of resistant and susceptible soybean varieties were studied by Endo (1965). In both cases, the syncytia were reported to be formed in 2-3 days after inoculation; however, most syncytia degenerated within 5 days after inoculation in resistant 'Peking'. The mode of tolerance to SCN is soybean has not been reported. The present study was done to examine syncytia development in tolerant PI 97,100 and compare it with that in susceptible 'Essex'.

Materials and methods: Two soybean lines, PI 97,100 and Essex were studied against SCN Race 3 population. Approximately 20,000 eggs of *H. glycines* were collected, mixed with moist seed, placed into a 4 cm deep cup, and stirred thoroughly. The radicle tips of three-day-old seedlings of each line were submerged into the sand to about 1.0 cm depth. After 24 hrs. incubation at 27°C, seedlings were lifted from the cup, sand was washed from the roots, and seedlings were transplanted in 7.5 cm clay pots filled with sterilized soil.

For sampling, plants were removed from the clay pots, roots were washed, and infected zones were excised using a razor blade. Samples were taken at 3, 8, and 18 days after inoculation (DAI). Root samples were kept in FAA fixative for two days and then dehydrated with tertiary butyl alcohol series and embedded in Tissuemat.

Cross-sections were cut at 10μ, affixed to slides by Haupt's gelatin adhesive and formalin and stained with safranin and fast-green. At least 5 root cross-sections were studied at each stage of development in both soybean lines. Syncytia were measured at the largest point of their attachment with the developing larvae.

Results and discussion: The width of syncytia at 3, 8 and 18 DAI are given in Table 1. At 3 DAI, there was no appreciable difference in the width of syncytia and PI 97,100 and 'Essex'. Eight DAI, the syncytia in Essex had enlarged to an average width of 131μ whereas, they remained approximately of the same size in PI 97,100. After 18 DAI, the syncytia in 'Essex' further increased in size and occupied large spaces in the vascular tissue of the roots. On the other hand, the syncytia in PI 97,100 remained small.

No significant differences were observed in the development or in the size of females in PI 97,100 or 'Essex'. It indicates that female juveniles obtained enough nutrients from small syncytia in PI 97,100 for normal development. It appears that tolerance to SCN in 97,100 is due to the development of small syncytia in the root tissue resulting in no appreciable yield loss to the plant.

Table 1. Width of Syncytia in PI97,100 and Essex (μ)

	Days After Inoculation		
Soybean line	3	8	18
PI97,100	47	58	54
Essex	64	131	197
LSD	18	35	48

References:

Anand, S. C., S. R. Koenning. 1986. Tolerance of soybean to Heterodera glycines. J. Nematol. 18:195-199.

Boerma, H. R., R. S. Hussey. 1984. Tolerance to Heterodera glycines in soybeans. J. Nematol. 16:289-296.

Endo, B. Y. 1965. Histological responses of resistant and susceptible soybean varieties, and backcross progency to entry and development of Heterodera glycines. Phytopathology 55:375-381.

S. C. Anand
A. K. Dwivedi
J. A. Wrather

UNIVERSITY OF MISSOURI
Department of Agronomy
Columbia, MO 65211

245

Inheritance of resistance in soybean accessions to Race 3 isolate of *Heterodera glycines*

Soybean cyst nematode (SCN), a destructive pest on *Glycine max* (L.) Merrill., was first reported in the USA in 1954 (Winstead et al., 1955). Soon after SCN was identified as a soybean pest, scientists sought resistance. Based on the reports, inheritance of resistance in soybean is complex. Earlier studies based on field populations of SCN Race 1 in North Carolina indicated that resistance in 'Peking' soybean was conditioned by a combination of three independent recessive genes: rhg_1, rhg_2, and rhg_3 (Caldwell et al., 1960). A dominant gene, Rhg_4, linked with the *i* locus for black-seed-coat color in Peking was found to be also required for resistance to SCN Race 3 field populations found in Missouri (Matson & Williams, 1965). A recent study indicated that resistance in Peking and PI90763 to (relatively 'homogeneous' populations) SCN Race 3 is conditioned by a combination of one dominant (Rhg_4) and two recessive genes (rhg_1, rhg_2) in each parent; whereas resistance in PI88788 is conditioned by a combination of two dominant (Rhg_4, Rhg) and one recessive (rhg_2) genes (Rao-Arelli et al., 1992). The purpose of this brief report is to provide information on the genetics of resistance in additional soybean accessions.

Materials and methods: The soybean genotypes utilized for this study included PI89772, PI404166, PI438489B and PI209332, which are resistant to SCN Race 3 isolate. Plants from each of the four different resistant accessions were crossed separately with a susceptible cultivar Essex to produce F_1 seed. The part of the F_1 seed was planted in Puerto Rico (off-season nursery) to generate F_2 seed. A sample of F_1 and F_2 plants from each cross were evaluated in the greenhouse at the University of Missouri-Delta Center for their reaction to SCN Race 3 isolate. For each cross 10 plants of each parent, five

F_1 plants, 288 to 339 F_2 plants and a set of standard differentials were evaluated against relatively homogeneous populations of SCN Race 3 isolate as described by Rao-Arelli and Anand (1988). The inoculation techniques used in this study were already reported (Rao-Arelli et al., 1991). Thirty days after inoculation plant roots were washed and the dislodged white females were counted using a stereo microscope.

In previous genetic studies of SCN resistance, plants have been classified as either highly resistance or susceptible based on the index of parasitism (IP). Resistance has been defined as <10% IP, following the classification standard established by Golden et al., 1970. In this report, we have defined resistance strictly based on the reaction of resistant parent i.e., range of cysts observed for 10 plants in each of the four different accessions. Thus in each cross, individual F_1 and F_2 plants with reaction for resistance similar to that of their respective parents, which is solely based on range of cysts obtained, were identified resistant. Chi-square analysis was used to test goodness of fit to appropriate genetic hypothesis.

Results and discussion: Means, ranges of cyst counts and variances for F_1 and F_2 plants of the four different crosses obtained in this report are provided in Table 1. The observed resistant vs. susceptible frequencies for F_1 and F_2 plants are also included in the same table, along with hypothesized resistance genes in each of the four resistant parents.

The F_1 hybrids of all four crosses were susceptible to SCN Race 3 isolate. Their means and ranges for all four crosses are below that of susceptible parent, Essex. This indicated incomplete dominance of Essex's susceptibilty over nematode resistance in the resistant parents used in this report. Similar results were already reported (Rao-Arelli et al., 1989).

The F_2 plants in each of the two crosses PI438489B x Essex and PI404166 x Essex, segregated closely to 1R:15S and 9R:55S, respectively. The remaining crosses, PI89772 x Essex and PI209332 x Essex, both segregated closely to 3R:13S in F_2 generation. This indicates that PI438489B has two major recessive genes, and PI494166 has two dominant genes plus one

recessive gene for resistance, respectively. PI89772 and PI209332 both have one dominant and one recessive gene for resistance to SCN Race 3 isolate.

We await data confirming our F_2 results and for confirmation of our values with and without index of parasitism.

References

Caldwell, B. E., C. A. Brim and J. P. Ross. 1960. Inheritance of resistance of soybeans to the cyst nematode, *Heterodera glycines*. Agron. J. 52:635-636.

Golden, A. M., J. M. Epps, R. D. Riggs, L. A. Duclos, J. A. Fox and R. L. Bernard. 1970. Terminology and identity of infraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Dis. Rep. 54:544-546.

Matson, A. L. and L. F. Williams. 1965. Evidence of a fourth gene for resistance to the soybean cyst nematode. Crop Sci. 5:477.

Rao-Arelli, A. P. and S. C. Anand. 1988. Genetic relationships among soybean plant introductions for resistance to Race 3 soybean cyst nematode. Crop Sci. 28:650-652.

Rao-Arelli, A. P., S. C. Anand and G. O. Myers. 1989. Partial dominance of susceptibility in soybean to soybean cyst nematode Races 3, 4 and 5. Crop Sci. 29:1562-1564.

Rao-Arelli, A. P., K. W. Matson and S. C. Anand. 1991. A rapid method for inoculating soybean seedlings with *Heterodera glycines*. Plant Dis. 75:594-597.

Rao-Arelli, A. P., S. C. Anand and J. A. Wrather. 1992. Soybean resistance to soybean cyst nematode Race 3 is conditioned by an additional dominant gene. Crop Sci. 34:862-864.

A. P. Rao-Arelli
P. A. Owen
G. U. Berger

Table 1. Mean number, range of cysts per plant obtained for parents, F_1's, and F_2's and their reaction to soybean cyst nematode Race 3 isolate in greenhouse.

Cross/genotype	Population	Resistant			Susceptible			Hypothesized resistance genes	x^2	Probability	Genetic ratio R:S
		Mean	Range	Variance	Mean	Range	Variance				
PI438489B x Essex	F_1				62.2	58-68	16.2				
	F_2	0.20	0-1	0.17	32.58	2-130	548.50	rr	0.085	0.70-0.80	1:15
PI404166 x Essex	F_1				57.4	48-62	29.8				
	F_2	0.27	0-1	0.20	39.23	2-191	1831.8	RRr	0.155	0.50-0.70	9:55
PI89772 x Essex	F_1				91.0	88-98	15.7				
	F_2	2.09	0-5	2.57	115.1	6-346	7010	Rr	0.042	0.80-0.90	3:13
PI209332 x Essex	F_1				91.2	82-99	49.2				
	F_2	2.88	0-7	6.8	73.9	8-340	3409.8	Rr	2.27	0.10-0.20	3:13
PI438489B (P_1)		0.3	0-1	0.23							
Essex (P_2)					118	91-136	41.0				
PI404166 (P_1)		0.2	0-1	0.18							
Essex (P_2)					182	156-194	21.0				
PI89772 (P_1)		1.1	0-5	2.54							
Essex (P_2)					140	128-320	4001				
PI209332 (P_1)		2.6	0-7	6.49							
Essex (P_2)					152	141-301	3409.8				

UNIVERSITY OF NEW HAMPSHIRE
Department of Plant Biology
Durham, NH 03824

245

Comparing three Chi-Square tests in detecting genetic linkages

In linkage studies, there are two methods for calculating recombination frequencies: the product method (Immer and Henderson, 1943) and the method of maximum likelihood (Allard, 1956). The maximum likelihood method provides the most accurate estimates of recombination frequencies for all kinds of data, whereas the product method is simple and convenient for 9:3:3:1 F_2 data. Either method requires, as a precondition, significant X^2 for deviations from independent assortment at the gene pair under study. Therefore, the Chi-square test is important. Currently there are three Chi-square tests available for linkage studies: the simple Chi-square, the Split Chi-square (Rao, 1983 and Yu and Kiang, 1990) and the Contingency Chi-square (Suiter et al., 1983). The simple X^2 is calculated with the expected frequencies derived from the sample size and the theoretical dihybrid segregation ratios, such as 9:3:3:1 in F_2. The expected frequencies of the Split Chi-square are calculated the same way as the simple Chi-square. The difference is that the portion of X^2 caused by deviations of monogenic segregations from the theoretical Mendelian ratios is removed from the total X^2, along with the monogenic degrees of freedom. The rest of X^2 and degrees of freedom is used in the linkage test. The Contingency Chi-square is quite different in that the expected frequencies are calculated from the sample size and the actual phenotypic frequencies of the two loci instead of the theoretical dihybrid ratios as in the other two methods. The degrees of freedom of the Contingency Chi-square are the product of the degrees of freedom of the two loci for monogenic segregations. This paper compares the three Chi-square methods with actual data.

Materials and methods: The F_2 interspecific segregation data involving the reciprocal crosses of AV68, the cultivated soybean [Glycine max (L.) Merr.] and KC13, wild soybean (Glycine soja Sieb. & Zucc.) were from Yu and Kiang (1993) and solely for comparisons of the three Chi-square tests. The simple, the Split and the Contingency X^2's were calculated for each isozyme gene pair. The sensitivities of the three tests can

be reflected by their probabilities of significance. The lower the probabilities, the stronger the deviations of gene pairs from independent assortment, and the higher sensitivity the Chi-square test. The probabilities were converted to arbitrary Probability Level Scores (PLS) so that the sensitivities of the three Chi-square tests could be easily visualized. The PLS and the probability (p) ranges they represent are as follows: 0, 1-0.995; 1, 0.994-0.990; 2, 0.989-0.975; 3, 0.974-0.950; 4, 0.949-0.900; 5, 0.899-0.750; 6, 0.749-0.500; 7, 0.499-0.250; 8, 0.249-0.100; 9, 0.099-0.050; 10, 0.049-0.025; 11, 0.024-0.010; 12, 0.009-0.005; 13, 0.004-0. The significance at the 95% and 99% probability levels is thus expressed by the PLS equal to or larger than 10 and 12, respectively.

Results and discussion: The X^2's obtained by the Split and the Contingency Chi-square methods were much lower than those by the simple Chi-square method for all gene pairs (Table 1). However, the sensitivities of the Split and the Contingency Chi-square tests expressed by the PLS were higher for most of the gene pairs. For example, Fle-Pgd1, Idh1-Idh2 and Pgd1-Ap gene pairs were not significant with the simple Chi-square method, but they were significant using the other two methods (PLS≥10). Obviously the increased sensitivities were mainly due to the decrease of degrees of freedom in the Split and the Contingency Chi-square tests. Here, the increased sensitivities are unwanted because we know that there are no genetic linkage in either Idh1-Idh2 or Pgd1-Ap. To encounter these unwanted higher sensitivities the 99% probability level is the reliable criterion and should be used in linkage studies. This conclusion is also supported by other data sets (Yu and Kiang, 1993).

Theoretically, the Contingency Chi-square is most suitable for detecting the association between loci. The Split Chi-square is an alternative. The simple Chi-square should never be used in studies of genetic linkages. However, the results of this study with actual data suggested that all these three Chi-square tests gave similar results if the 99% probability level was used for the Split and the Contingency Chi-square tests.

References:
Allard, R. W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235-278.
Immer, F. R., M. T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.
Rao, G. N. 1983. Statistics for Agricultural Sciences. Oxford & IBH Publishing Co., New Delhi, India.
Suiter, K. A., J. F. Wendel, J. S. Case. 1983. Linkage-1: a Pascal computer program for the detection and analysis of genetic linkage. J. Hered. 74:203-204.
Yu, H., Y. T. Kiang. 1990. Re-examination of the recombination frequency between acid phosphatase and the Kunitz trypsin inhibitor loci in soybean. Soy. Genet. Newsl. 17:106-108.
Yu, H., Y. T. Kiang. 1993. Inheritance and linkage studies of isozyme loci in soybean (submitted to J. Hered.).

H. Yu
Y. T. Kiang

Table 1. Three Chi-square tests on possible linkages among all comparisons of 11 isozyme loci in F_2 data.

Gene pair[a]	Observed frequencies[b]									Simple		Split		Contingency	
	e	f	g	h+i	j	k	l	m	n	x^2/df	PLS	x^2/df	PLS	x^2/df	PLS
Aco2-Ap	16	47	37	73	16	39	18	35	19	3.53/8	5	1.73/4	5	1.64/4	5
Aco2-Dia1	15	37	42	77	12	45	18	37	17	5.48/8	6	3.75/4	7	3.97/4	7
Aco2-Enp	17	40	36	83	16	36	17	38	17	2.04/8	2	0.08/4	0	0.07/4	0
Aco2-Idh1	19	42	38	86	12	31	19	37	16	6.13/8	6	0.44/4	2	0.55/4	3
Aco2-Idh2	18	36	29	88	22	35	20	33	19	5.63/8	6	4.46/4	7	4.53/4	7
Aco2-Lap1	13	38	37	87	19	34	18	39	15	4.87/8	5	1.47/4	5	1.66/4	5
Aco2-Pgm2	21	47	32	80	16	32	17	32	23	6.96/8	6	4.03/4	7	4.10/4	7
Ap-Dia1	13	33	40	75	21	37	24	41	16	4.95/8	5	3.70/4	7	3.79/4	7
Ap-Enp	20	33	38	77	16	35	21	42	18	2.10/8	2	0.62/4	3	0.63/4	3
Ap-Idh1	19	38	40	80	15	27	23	41	17	5.65/8	6	0.44/4	2	0.50/4	3
Ap-Idh2	18	37	38	73	18	35	19	39	23	1.32/8	0	0.63/4	3	0.59/4	3
Ap-Lap1	1	23	35	97	38	25	45	31	5	100.92/8	13	98.00/4	13	102.81/4	13
Ap-Pgm2	22	47	39	62	13	36	16	43	22	8.88/8	7	6.43/4	8	6.28/4	8

Dia1-Enp	16	38	40	76	18	42	20	41	9	6.64/8	6	5.23/4	7	5.90/4	8
Dia1-Idh1	17	36	39	93	18	27	27	29	14	14.33/8	9	9.19/4	9	9.11/4	9
Dia1-Idh2	25	30	37	83	12	43	19	30	21	9.45/8	7	8.83/4	9	8.91/4	9
Dia1-Lap1	16	31	36	93	22	32	22	34	14	9.37/8	7	6.52/4	8	6.72/4	8
Dia1-Pgm2	17	51	42	66	15	39	17	36	17	7.48/8	7	5.46/4	8	5.10/4	7
Enp-Idh1	13	28	44	86	17	43	18	31	20	9.12/8	7	3.75/4	7	3.91/4	7
Enp-Idh2	20	39	33	86	21	32	17	31	21	4.93/8	5	4.08/4	7	4.13/4	7
Enp-Lap1	16	43	40	80	18	34	9	43	17	8.11/8	7	5.03/4	7	5.80/4	8
Enp-Pgm2	22	31	32	82	20	44	18	30	21	6.16/8	6	3.55/4	7	3.73/4	7
Eu-Aco2		55		124		58	17	35	11	4.79/5	7	1.09/2	6	1.46/2	7
Eu-Ap		67		118		52	14	27	22	7.35/5	8	4.13/2	8	4.57/2	8
Eu-Dia1		53		124		60	17	32	14	3.79/5	6	0.64/2	6	0.67/2	6
Eu-Enp		58		122		57	16	35	12	3.87/5	6	0.49/2	5	0.72/2	6
Eu-Fle				177		60		44	19	3.32/3	7	0.48/1	7	0.60/1	7
Eu-Idh1		63		125		49	17	36	10	7.44/5	8	0.33/2	5	0.77/2	6
Eu-Idh2		60		118		59	14	32	17	2.83/5	6	0.24/2	3	0.29/2	5
Eu-Lap1		56		132		49	13	31	19	7.21/5	8	2.39/2	7	2.55/2	5
Eu-Pgd1				180		57		41	22	5.46/3	8	2.62/1	8	3.03/1	9
Eu-Pgm2		71		116		50	14	28	21	8.55/5	8	4.20/2	8	4.41/2	8
Fle-Aco2		54		120		47	18	39	22	2.76/5	6	1.34/2	6	1.43/2	7

Fle-Ap		64		100		57	17	44	18	3.79/5	6	2.85/2	8	2.74/2	7
Fle-Dia1		53		115		53	17	41	21	1.19/5	4	0.32/2	5	0.31/2	5
Fle-Enp		57		114		50	17	43	19	1.70/5	5	0.60/2	6	0.57/2	5
Fle-Idh1		59		123		39	21	38	20	6.76/5	8	1.93/2	7	2.36/2	7
Fle-Idh2		54		116		51	20	34	25	3.18/5	6	2.87/2	8	2.74/2	7
Fle-Lap1		56		113		52	13	50	16	6.66/5	8	4.12/2	8	3.86/2	8
Fle-Pgd1				156		65		65	14	4.89/3	8	4.33/1	10	4.10/1	10
Fle-Pgm2		58		114		49	27	30	22	6.70/5	8	4.63/2	9	4.33/2	8
Idh1-Idh2	10	50	34	77	15	34	14	39	27	14.60/8	9	10.02/4	10	9.98/4	10
Idh1-Lap1	18	33	29	88	12	40	18	46	16	9.71/8	7	2.90/4	6	3.06/4	6
Idh1-Pgm2	13	48	27	77	19	36	24	40	16	9.80/8	7	3.46/4	7	3.55/4	7
Idh2-Lap1	12	38	43	80	19	32	18	40	18	4.61/8	5	2.32/4	6	2.55/4	6
Idh2-Pgm2	19	36	33	76	22	38	16	35	25	3.84/8	5	2.02/4	6	1.90/4	5
Lap1-Pgm2	16	44	37	72	16	47	11	35	22	8.40/8	7	4.35/4	7	4.55/4	7
Pgd1-Aco2		55		119		47	14	40	25	5.38/5	7	3.96/2	8	4.02/2	8
Pgd1-Ap		46		115		60	28	30	21	8.51/5	8	7.57/2	11	7.54/2	11
Pgd1-Dia1		54		116		51	20	40	19	0.94/5	3	0.07/2	3	0.08/2	3
Pgd1-Enp		52		116		53	17	41	21	1.38/5	4	0.28/2	5	0.27/2	5
Pgd1-Idh1		47		122		52	12	39	28	9.70/5	9	4.87/2	9	4.56/2	8

Pgd1-Idh2	59	107	55	17	43	19	1.40/5	4	1.09/2	6	1.05/2	6
Pgd1-Lap1	51	119	51	17	44	18	2.68/5	6	0.14/2	4	0.10/2	4
Pgd1-Pgm2	55	105	61	16	39	24	2.86/5	6	0.79/2	6	0.73/2	6

---

[a] N = 300 for all gene pairs; All gene pairs were either in or organized into repulsion phase except for Eu-Fle, which is in coupling phase.

[b] Classification of genotypes into e to n classes was per Allard (1956). The theoretical segregation ratios for the nine, six and four category data are 1:2:2:4:1:2:1:2:1 [e:f:g:(h+i):j:k:l:m:n], 3:6:3:1:2:1 [(e+f):(g+h+i):(j+k):l:m:n] and 9:3:3:1 [(e+f+g+h+i):(j+k):(l+m):n or a:b:c:d], respectively.

USDA-ARS SOIL DRAINAGE RESEARCH AND
THE OHIO STATE UNIVERSITY
Columbus, Ohio 43210
in collaboration with the
Shanghai Institute of Plant Physiology
Academia Sinica, Shanghai, China 200032[1]

245

RAPD Markers of Flooding Tolerant Chinese Soybean Germplasm

Introduction: Soybean is generally tolerant to environmental stresses, with the exception of drought and flooding. In Ohio, soybean yield loss due to flooding in excessively wet years (e.g. 1990 and 1992) was estimated to be as high as 15% (A. F. Schmitthenner, per. com.). American soybean has a very narrow genetic base (Delannay et al., 1983) and is fairly uniform in its susceptibility to flooding (VanToai, unpublished data). The soybean landraces grown on dikes of paddy rice fields in southeastern China are apparently more tolerant to flooding, but their tolerance level has never been investigated.

The objectives of this study were to evaluate the flooding tolerance of soybean germplasm from southeastern China and to fingerprint the molecular markers of flooding tolerance using the newly developed Random Amplified Polymorphic DNA (RAPD) technique (Williams et al., 1991).

Materials and methods:

Plant Materials. Seeds of three Chinese soybean landraces, 'Baimongjie', 'Dabingchin', 'Xu 89-2', and of two American cultivars, 'Williams' and 'Williams 82' were surface sterilized and germinated in paper rolls as described by Hwang and VanToai (1991). Healthy, unblemished 3-d-old seedlings were sorted for uniformity in size before being used in the experiments.

Screening for Flooding Tolerance. A simulated flooding stress was imposed for 48 h as described by Hwang and VanToai (1991) except that degassed buffer (5 mM Tris-HCl, pH 8.0 and 100 mg/L ampicillin) was used instead of distilled water (Sachs and Freeling, 1978). The viability results were

the averages of three independent experiments, each with three replications of 50 seedlings per treatment.

DNA Extraction. Soybean genomic DNA was extracted from leaf discs (10-15 mg) using the procedure of Luo et al. (1992). The supernatant contained 12-18 ng DNA/μl.

RAPD Primers. The oligonucleotide decamers (set #5) were obtained from Dr. John Carlson, University of British Columbia, Vancouver, Canada. The primers were synthesized using a PCR-MATE DNA synthesizer (Applied Biosystem Inc.)[2] and purified with NAP-5 (Pharmacia) disposable columns.

RAPD Reaction. Each reaction (25 μl volume) was performed in 0.5 ml microfuge tubes. The final concentrations of the reaction components were: 1.0-1.5 ng/μl DNA template, 0.1 unit/μl Taq DNA polymerase (Perkin Elmer), 10 mM Tris-HCl (pH 8.3), 90 mM KCl, 3.0 mM $MgCl_2$, 0.001% gelatin, 0.2 mM dNTP, 0.2-0.4 μM primer. The reaction was covered with 20 μl mineral oil and subjected to 40 PCR cycles in a MiniCycler (MJ Research, Inc.). The thermal profile was 1 min at 94°C, 1 min at 40°C and 1 min at 72°C.

Electrophoresis. The PCR products were analyzed by 2% agarose electrophoresis (Maniatis et al., 1982).

Results and discussion:

Flooding tolerance of Chinese soybean germplasm. After 48 h of submersion in degassed buffer, the average survival of 'Williams' and 'Williams 82' was 58% and 61%, respectively (Table 1). Among the three Chinese landraces, 'Baimongjie' showed similar flooding survival (66%) with 'Williams' and 'Williams 82'; 'Xu 89-2' showed the highest flooding survival at 84% and 'Dabingchin', the lowest survival at 14%. In our screening of soybean genotypes for flooding tolerance, 'Xu 89-2' appears to be the best. However, the difference in flooding survival between 'Xu 89-2' and 'Williams' and 'Williams 82' was only about 10%. 'Dabingchin' appears very susceptible to flooding stress. The low flooding survival of this genotype could have been due, at least in part, to the low vigor of the seed lot (51% germination).

Polymorphism of RAPD markers among Chinese and American soybean. Of the 40 RAPD primers screened, 23 produced at least one polymorphism among the five genotypes, 4 produced no polymorphism, and 13 failed to amplify any discernible bands. Among the primers that generated DNA bands, 85% detected at least one polymorphic locus. This ratio was rather high, given the common belief that there is relatively low polymorphism among soybeans due to their lack of genetic diversity. While the majority of the primers (43%) showed only 1 polymorphic band, as many as 6 polymorphic bands were detected by two primers (Fig. 1A and 1B).

The RAPD primers in this study generally amplified more DNA bands from the Chinese soybean than from the American soybean (Fig. 1A, 1B and 1D). These results might reflect the more homogeneous genetic makeup of the American soybean. As a group, the Chinese soybeans usually produced RAPD fingerprints that were distinctly different from those produced by the American soybean (Fig. 1A, 1B, 1C, and 1D). Among the Chinese soybeans, fingerprints of 'Dabingchin', the vegetable soybean, were also different from those of 'Baimongjie' and 'Xu 89-2' genotypes. Markers that are specific for 'Xu 89-2' were detected by several primers (i.e. Fig. 1A, bank 780). While 'Xu 89-2' is more tolerant to flooding than the other soybean genotypes, the relationship between these RAPD markers and the flood-tolerance trait remains to be tested by segregation analyses. Polymorphism is usually qualitative: a band is either present or not, but non stoichiometric differences in band intensity were also detected (Fig. 1D, lanes b, c, and d). The significance of the difference in band intensity can be determined when the amplified DNA fragments are cloned and sequenced.

Markers that are specific for either 'Williams' or 'Williams 82' were also detected in this study (Fig. 1A and 1B). 'Williams 82' is an isoline of 'Williams' that contains the *rps1K* gene. The probability that these markers are associated with the *rps1* gene exists and remains to be tested.

The reproducibility of the RAPD technique tested on a number of DNA templates and primers was close to 100%. The RAPD technique can identify

more polymorphisms than any other type of genetic or molecular markers. Compared with RFLP, the RAPD technique is much simpler, faster, less expensive, and does not require the use of radioactive isotopes. The RAPD technique is very valuable tool for the mapping of quantitative trait loci.

References:

Delannay, X., D. M. Rodgers, R. G. Palmer. 1983. Relative genetic contributions among ancestral lines to north American soybean cultivars. Crop Science 23:944-949.

Hwang, S. Y., T. T. VanToai. 1991. Abscisic acid induces anaerobiosis tolerance in corn. Plant Physiol. 97:593-597.

Luo, Guangbin, A. G. Hepburn, J. M. Widholm. 1992. Preparation of plant DNA for PCR analysis: A fast, general and reliable procedure. Plant Molecular Biology Reporter 10:319-323.

Maniatis, T., E. F. Fritsch, J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, S. V. Tingey. 1991. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

[1]This research is supported by a grant from USDA-OICD.

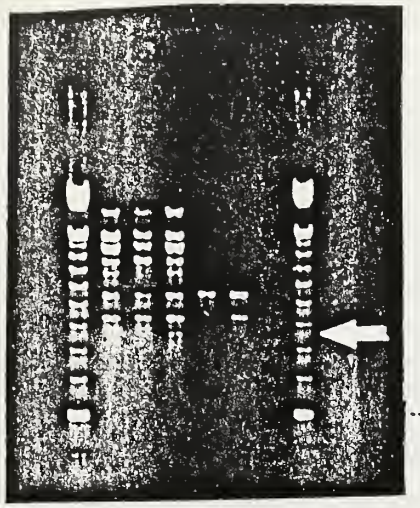
[2]Use of trade names is for the benefit of the readers and does not imply endorsement of the product by the USDA.

Tara T. VanToai
Steven K. St. Martin
Jianhuan Zhang
Shu-wen Yu

Figure 1. RAPD fingerprints of some Chinese and American soybeans. Numbers on the right indicate molecular weight. Arrow shows the polymorphic band in Xu 89-2.

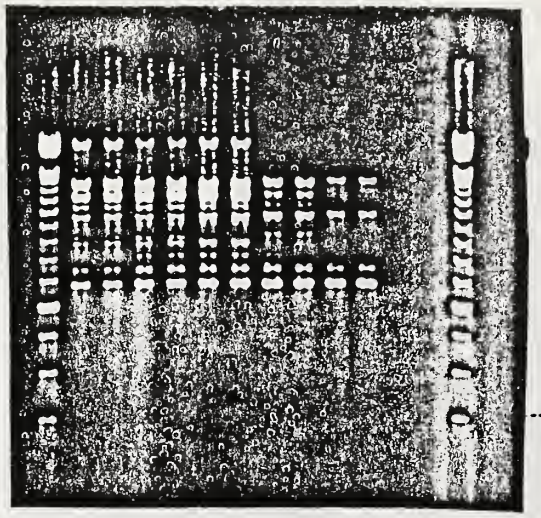
1A. 1C and 1D. Lane 1, molecular weight markers; Lane 2, Baimongjie; Lane 3, Dabingchin; Lane 4, Xu 89-2, Lane 5, Williams; Lane 6, Williams 82; Lane 7, control, no DNA template; Lane 8, molecular weight markers. **1B.** Lane 1, molecular weight markers; Lanes 2 & 3, Baimongjie; Lanes 4 & 5, Dabingchin; Lanes 6 & 7, Xu 89-2; Lanes 8 & 9, Williams; Lanes 10 & 11, Williams 82; Lanes 12 & 13, control, no DNA template; Lane 14, molecular weight markers.

Figure 1A



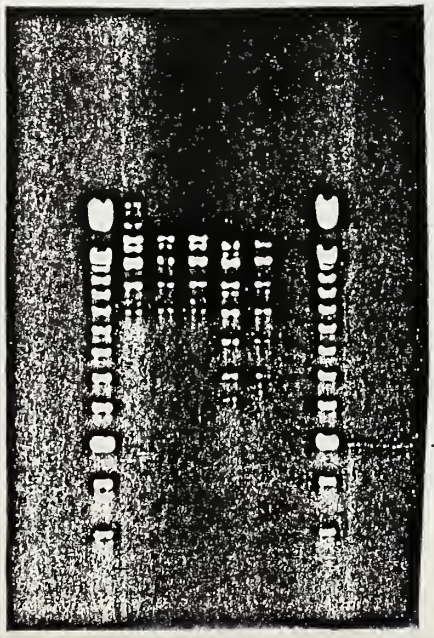
500

Figure 1B



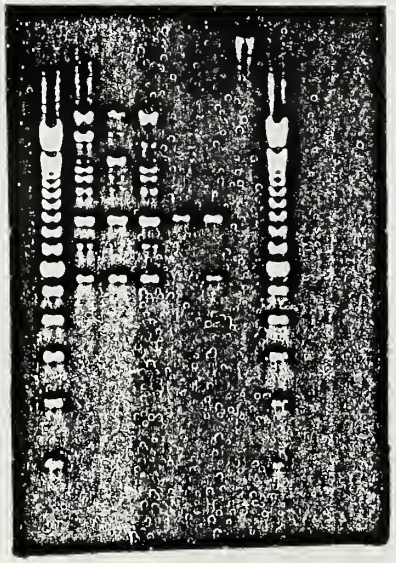
100

Figure 1C



600

Figure 1D



100

Table 1: Flooding survival of soybean seedlings

Genotypes	Germination (%)	Survival (%)	
		Control	Flooded
Baimongjie	91 ± 6	85 ± 11	66 ± 7
Dabingchin	51 ± 20	37 ± 9	14 ± 10
Xu 89.2	88 ± 3	97 ± 1	84 ± 5
Williams	97 ± 3	99 ± 1	58 ± 6
Williams 82	97 ± 3	99 ± 1	61 ± 8

UNIVERSITY OF RHODE ISLAND
Department of Plant Sciences
Kingston, RI 02881

245

The recessive mutation (y18) at the Y18 locus results in the loss of a component of the oxygen evolving complex in photosystem II

Y18-m, an unstable allele at a gene affecting chloroplast development in the soybean, has been subject to analysis in this laboratory for the past several years. Previous genetic studies have localized this unstable allele to a single nuclear gene locus (Y18; Peterson and Weber, 1969). Three alleles have been identified: Y18 - the dominant allele, y18 - the recessive allele, and Y18-m - the unstable allele. Somatic mutability of the unstable allele produces variegated green/yellow sectors in leaves at all stages of development (Chandlee and Vodkin, 1989a,b). Germinal mutations produce either pure breeding green (rare) or pure breeding yellow (frequent) plants from the original mutable stock (Peterson and Weber, 1969). The homozygous yellow plants (y18,y18) are lethal, but can survive to maturity under reduced light conditions. The instability of the Y18-m allele is affected by temperature (Sheridan and Palmer, 1977). The pattern of mutation is in the direction of a dominant (Y18-m) to a recessive (y18) form, analogous to the "reverse variegation" patterns observed in several other systems (Vodkin, 1988).

The chloroplasts found in mutant yellow tissue from mutable plants have a reduced chlorophyll content (reduced by half), but the ratio of chlorophyll a and b remains the same. The thylakoid membrane structure within the defective chloroplasts also is degenerated (Palmer et al., 1979). In addition, several thylakoid membrane proteins are missing or reduced (Chandlee and Vodkin, 1989a). Transcripts of two nuclear-encoded chloroplast genes (rbcS and Cab) are reduced from their wild type expression levels by 70% and 90%, respectively. In order to better understand the specific genetic lesion conditioned by this mutant allele, we have subjected the mutable plants to further analysis. The following is a summary of our observations.

1. Ultrastructural and biochemical analysis indicates that the photosynthetic capability of defective chloroplasts from yellow tissue is decreased by the mutation through an effect on the photosynthetic complexes of the thylakoid membrane, specifically

on photosystem I (PSI) and photosystem II (PSII). Fluorescence microscopy analysis revealed lower levels of photosynthetic activity within the yellow sectors. Since fluorescence levels are primarily indicative of chlorophyll associated with PSII (Malkin, 1977), these data suggest that PSII complexes are affected by the y18 mutation. Moreover, spectrophotometric analysis of photosynthetic pigments in conjunction with mild LDS-PAGE (green gel) analysis indicates that chloroplasts from homzygous y18 mutant tissue exhibit qualitatively similar profiles for individual photosynthetic pigments and individual photosynthetic complexes. Pigment-protein complexes corresponding to CP1 (p700-chl a complex from PSI), $LHCP^1$ (light harvesting chlorophyll a/b binding protein II from PSII, oligomeric LHCIIb), $LHCP^2$ (dimeric LHCIIb), CPa (chlorophyll a-protein from PSII), $LHCP^3$ (monomeric LHCIIb), and free pigment (FP) are maintained, but present in much lower levels in the mutant chloroplasts.

2. A pleiotropic reduction, or loss of several thylakoid membrane proteins was observed in chloroplasts from y18 mutant tissue. In order to determine if one of the missing polypeptides represents the primary defect in homozygous y18 tissues (i.e., the Y18 gene product), experiments were conducted using in vitro translation coupled with an in vitro chloroplast uptake system. In vitro translation products produced from mRNA isolated from either green or yellow tissue were added to an in vitro reconstitution system containing freshly isolated chloroplasts from green tissue. After an incubation period, thylakoid membranes were isolated from the chloroplasts and analyzed by LDS-PAGE. By this approach it was determined that a polypeptide with a molecular weight of ~ 18 kD was absent in the products translated and processed from the mRNA from yellow tissue. This result, coupled with the evidence indicating a reduction in PSII complexes in mutant chloroplasts, suggested a specific defect in a product of PSII with a molecular weight of about 18 kD. Three protein components in the oxygen-evolving complex (OEC) of PSII are OEC33 (33 kD), OEC23 (23 kD) and OEC16 (16 kD) which are encoded by the nuclear genes oee1, oee2, and oee3, respectively (Andersson, 1986). Available antibodies and gene clones allowed us to examine the expression and accumulation of these products in normal and mutant leaf tissue. Western blot analysis revealed that OEC16 is not detectable in thylakoid membranes of chloroplasts from y18 mutant yellow tissue, whereas the other two components, OEC23 and OEC33, are present.

3. In order to determine how the y18 mutation prevents the accumulation of the OEC16 polypeptide and to determine the effects of its absence on OEC23 and OEC33 gene expression, mRNAs encoding the three polypeptides were measured by Northern blots. The mRNA's for OEC16, 23, and 33 were all found to be expressed in mutant tissue at similar levels to that observed for non-mutant tissue. Therefore, transcription of the oee1 and oee2 genes is not affected by this nuclear mutation and the absence of OEC16 polypeptide in y18 mutant is not due to a block of transcription of the oee3 gene. Furthermore, the OEC16 mRNA isolated from y18 mutant tissue can be translated by a rabbit reticulocyte lysate in vitro translation system as determined by immunoprecipation experiments; however, the translation efficiency is lower than the OEC16 mRNA isolated from green tissue. This suggested that a translational and/or post-translational defect influences OEC16 protein accumulation in the mutant.

4. Experiments were designed to determine if the y18 mutation has an effect on chloroplast gene expression. Both psaA (the chloroplast gene encoding the P700 apoprotein A1 from PSI) and psbA (the chloroplast gene encoding the 32 kD protein from PSII) expression are apparently not affected by the mutation; however, rbcL (the chloroplast gene encoding the ribulose-1,5-bisphosphate carboxylase large subunit-Rubisco) and 16S rRNA transcripts are reduced in the chloroplasts of mutant yellow tissue. The reduction in rbcS (Rubisco small subunit) gene expression in the mutant has been reported previously by Chandlee and Vodkin (1989a). Therefore, the coordination between the nucleus and the chloroplast genetic compartments may not be affected by the mutation at least in regard to rbcS and rbcL. The reduction in the levels of chloroplast 16S rRNA in the mutant indicates that the translational machinery within the yellow chloroplasts may be affected. The significance of this is presently unknown.

In order to elucidate the effect of this mutation on soybean chloroplast development, attempts are underway to obtain cDNA and genomic clones of the oee3 gene derived from DNA from green and yellow leaf tissue. Comparisons should aid in determining an association between OEC16 and the specific defect in y18 mutant tissue.

References:

Andersson, B. 1986. Proteins participating in photosynthetic water oxidation. In: "Photosynthesis III. Encyclopedia of Plant Physiology, Vol. 19", Staehelin, L. A. and Arntzen, C. J., eds., Springer-Verlag, Berlin, pp.447-456.

Chandlee, J. M., L.O. Vodkin. 1989a. Unstable genes affecting chloroplast development in soybean. Developmental Genetics 10:532-541.

Chandlee, J. M., L.O. Vodkin. 1989b. Analysis of the Y18 gene of soybean and its associated alleles. Soybean Genetics Newsletter 16:199-201.

Malkin, S. 1977. Delayed luminescence. In: "Topics in Photosyanthesis", Barber, J., ed., Elsevier, Amsterdam, Netherlands, pp. 349-432.

Palmer, R. G., M.A. Sheridan, M.A. Tabatabai. 1979. Effects of genotype, temperature, and illuminance on chloroplast ultrastructure of a chlorophyll mutant in soybeans. Cyatologia 44:881-891.

Peterson, P. A., C.R. Weber. 1969. An unstable locus in soybeans. Theoretical and Applied Genetics 39:156-162.

Sheridan, M. A., R.G. Palmer. 1977. The effect of temperature on an unstable gene in soybeans: Mutant effects at the Y18-m locus at two temperature levels. The Journal of Heredity 68:17-22.

Vodkin, L. O. 1988. Transposable element influence on plant gene expression and variegation. In: "The Biochemistry of Plants/Plant Molecular Biology, Vol. 15", Marcus, A., ed., Academic Press, New York, NY, pp. 83-132.

Tai-Sheng Cheng
Joel M. Chandlee

CLEMSON UNIVERSITY
Division of Agriculture and Natural Resources
College of Agricultural Sciences
Department of Agronomy and Soils
Clemson, South Carolina

24

Tetramers in Soybean:

Several tetrameric enzymes were identified in plants. When conditioned by a single locus, a homotetramer AAAA or BBBB is expected in homozygous plants. In heterozygotes, two homotetramers (AAAA and BBBB) and three heterotetramers (AAAB, AABB, ABBB) are observed. Genetics of tetramers in soybean reflects tetraploid origin and it is complicated by multiple gene number. Diaphorase DIA (E.C. 1.6.4.3.), fructose-bisphosphate aldolase FBA (E.C. 4.1.2.13), fructose-bisphosphatase FBP (E.C. 3.1.3.11), glyceraldehyde-3-phosphate dehydrogenase ($NADP^+$)G3PDH (E.C. 1.2.1.9), malic enzyme ME (EC 1.1.1.38), were detected in soybean.

In homozygous germplasms of soybean, multibanded patterns were described for tetrameric enzymes. The genetic basis for the majority of multibanded patterns is unknown. Some isozymes generate five-banded clusters in homozygous lines suggesting two different loci, each of them producing a subunit which might interact and form intra- and interlocus tetramers. The variant patterns, which lack bands or the altered mobility of some bands, were also reported in the tetramer patterns in soybean, (Doong and Kiang, 1987; Palmer and Kilen, 1987; and Rennie et al., 1989).

We detected the "null" variant at the slow locus of ($NADP^+$)G3PDH. G3PDH was resolved on L-histidine-citrate (pH 5.7) buffer system and isozyme assay followed procedure described by Wendel and Weeden (1989). The genetics of this enzyme was determined in the F_3 generation. The analysis was conducted using $F_{2:3}$ progeny lines (4 seed/F_2 plants) from a Peking and Essex cross. Peking has three-banded pattern and Essex five-banded pattern. The intermediate phenotype resulting from gene doses of both parents was difficult to score due to invisibility of the slowest band and the intermediate pattern resembled Peking phenotype. The $F_{2:3}$ progeny segregated for lines expressing Peking pattern, lines segregating for ($NADP^+$)G3PDH patterns, and lines with

Essex pattern. The segregation ratio gave a good fit to a 1:2:1 ratio, x^2 = 2.5871 (Table 1), indicating codominance at the slower locus of ($NADP^+$)G3PDH. The "null" allelic variant of G3PDH functioned electrophoretically as a null only as hometetramer. We used the detected polymorphism at the slow locus of ($NADP^+$)G3PDH to look for association with morphological loci resulting from anthocyanin biosynthesis in the Peking x Essex cross in the F_3 generation. The flower color - W1 locus, pubescence color - T locus, and seed coat color - I locus were analyzed. The maximum likelihood method was used in calculating recombinational frequences (Suiter et al., 1987). No linkages were detected between the slow locus of ($NADP^+$)G3PDH and W1, T and I loci (Table 2). This eliminates the localization of the slow locus for ($NADP^+$)G3PDH in the tested chromosomal regions on the linkage groups 1, 7, and 8 of the soybean genetic map.

Cerff (1982) investigated the evolutionary relationships of thirteen angiosperm species, relevant to the G3PDH protein mobilities. He reported the rather rapid evolutionary changes of the ($NADP^+$)G3PDH during 200-300 million years of angiosperm speciation and concluded that the interspecific heterogeneity in subunit size and number may reflect the genetic changes resulting from insertion, deletions, possible gene duplication, and differential processing during the post-translational transport of the cytoplasmatically synthesized precursors into the chloroplasts. Therefore, soybean ($NADP^+$)G3PDH might be expected to show evolutionary rearrangements in the genus Glycine.

The ($NADP^+$)G3PDH is the second enzyme in the photosynthetic pathway after the fixation of carbon by ribulose-1, 5-bisphosphate carboxylase-oxygenase (RuBisCO) and converts 1,3 diphosphoglycerate to glyceraldehyde 3-phosphate. It is part of a five enzyme complex located in the chloroplast and comprised of RuBisCO, phosphoribulokinase (PRK), ribosephosphate isomerase (RPI), phosphoglycerate kinase (PGK). Although the enzymatic pathway of photosynthesis has been well characterized, only two enzymes, RuBisCO and sucrosephosphate synthetase (SPS), have been shown to regulate the net production of photosynthate in a crop species (Murthy and Singh, 1979; Losa-Tavera et al., 1987 and 1990; Rocher et al., 1989). However specific enzyme alleles might interact differentially with other enzymes to create the favorable condition for evolution and selection in photosynthesis and other metabolic pathways. The genetic

patterns of tetramers in soybean offer opportunities to explore gene expression, gene regulation, species diploidization, and interaction of duplicated loci. Molecular mapping of soybean genome might be helpful to resolve genetics of complex phenotypes of tetrameric enzymes and determine their importance in physiological efficiency of soybean.

References:

Cerff, R. 1982. Evolutionary divergence of chloroplast and cytosolic glyceraldehyde-3-phosphate dehydrogenases. Eur. J. Biochem. 126:513-515.

Doong, J. Y. H., T. Y. Kiang. 1987. Cultivar identification by isozyme analysis. Soy. Genet. Newsl. 14:189-226.

Losa-Tavera, H., B. Serrano, J. D. Molina, M. L. Ortega-Delgado, E. Sanchez-de-Jimenez. 1987. CO_2-fixation enzymes in maize (Zea mays) and grain yield. Can. J. Bot. 65:607-611.

Losa-Tavera, H., E. Martinez-Bajaras, E. Sanchez-de Jimenez. 1990. Regulation of ribulose-1, 5-bisphosphate carboxylase expression in second leaves of maize seedlings from low and high yield populations. Plant Physiol. 93:541-548.

Murthy, K. K., M. Singh. 1978. Photosynthesis, chlorophyll content and ribulose diphosphate carboxylase activity in relation to yield in wheat genotypes. J. Agric. Sci. Camb. 93:7-11.

Palmer, R. G., T. C. Kilen. 1987. Qualitative genetics and cytogenetics. In: Soybeans, production and uses. Second ed. (J. R. Wilcox, ed.). Agronomy 16. ASA, Inc., CSSA, Inc., SSSA, Inc. Publ., Madison, WI, USA.

Rennie, B. D., M. L. Thorpe, W. D. Beversdorf. 1989. A procedural manual for the detection and identification of soybean Glycine max (L.) Merr. isozymes using the starch gel electrophoretic system. Tech. Bull. OAC Publication, Univ. of Guelph, Ontario, Canada.

Rocher, J. P., J. L. Prioul, A. Lecharny, A. Reyss, M. Joussaume. 1989. Genetic variability in carbon fixation, sucrose-p-synthase and ADP glucose pyrophosphorylase in maize plants of differing growth rate. Plant Physiol. 89:416-420.

Suiter, K. A., J. F. Wendel, J. S. Case. 1983. Linkage-1. J. Heredity. 74:289-291.

R. Mahalingam
R. M. Delorme
H. T. Skorupska

Table 1. Inheritance of (NADP+)G3PDH "null" variant.

	Pattern*		
Number	A	Heterozygote	B
Population I			
Observed	30	49	23
Expected (1:2:1)	25.5	51	25.5
X^2 = 1.1175, df = 2, p > 0.50			
Population II			
Observed	45	79	31
Expected (1:2:1)	38.75	77.5	38.75
X^2 = 2.5871, df = 2, p > 0.10			

* Pattern described for $F_{2:3}$ lines; A - Peking pattern; B - Essex Pattern.

Table 2. Linkage test of the slow locus of (NADP+)G3PDH with morphological loci.

Locus Tested*	Ratio	df	n	x^2	r ± SE
W1	1:2:1 x 1:2:1	4	97	5.4495	49.9 ± 5.1
T	1:2:1 x 1:2:1	4	93	4.4973	47.5 ± 5.2
I	1:2:1 x 1:2:1	4	93	5.5850	46.5 ± 5.2

* Segregation was tested for $F_{2:3}$ progeny lines.

CLEMSON UNIVERSITY
Department of Agronomy and Soils
Poole Agricultural Center Box 340359
Clemson, SC 29634-0359
and
USDA/ARS-UNIVERSITY OF FLORIDA
P.O. Box 110790
Gainesville, FL 32611-0790

245

Growth and developmental responses of long-juvenile soybean genotypes at early and conventional planting dates

Introduction: Long-juvenile (LJ) soybean genotypes contain a photoperiod response character that delays flowering and maturity under short day conditions (Hartwig and Kiihl, 1979). At early plantings, conventional soybean cultivars may initiate flowering too soon for optimum production due to short day conditions. LJ genotypes, however, can provide improved yields at early planting dates over conventional types due to the delay in flowering that allows extended vegetative growth (Board and Hall, 1984).

Although the LJ flowering response across planting dates has been well characterized (Sinclair and Hinson, 1992), growth and developmental parameters associated with morphology and seed yield distribution have not. Board (1985) and Board and Settimi (1986) reported yield component and morphological responses of an LJ genotype across planting dates while Parvez and Gardner (1987) reported morphological responses of two LJ genotypes across planting dates. These studies, however, did not include a diverse array of LJ genotypes. Therefore, a preliminary study was undertaken to better characterize the LJ phenotype across planting dates using a greater number of LJ genotypes.

Materials and methods: Forty-two diverse LJ experimental lines, 1 LJ cultivar, and 8 conventional cultivars were planted in 4 row (76 cm apart) plots 6 m long at a rate of 28 seed m^{-1}. Plantings were performed 14 April (mid-April) and 28 May (late May), 1992, at Blackville, SC (Latitude 33°20'). For the southeastern U.S.A., these planting dates are considered to be very early and conventional, respectively. Eight LJ and three

conventional entries were selected for morphological and yield component analyses based on good emergence, uniformity of plant population, and genetic background with each LJ entry representing a different cross. The experimental design was a randomized complete block factorial with test entries randomly assigned and replicated twice within each of two planting dates. Individual plants were collected by randomly sampling six plants from each plot. Measurements taken for each plant were main stem length, total branch length, number of branches, main stem yield, and total branch yield. Data from each planting date were analyzed separately with analysis of variance procedures, and combined data across planting dates were analyzed using general linear model procedures. Experimental LJ lines F90-5614, F90-6908, F90-6293, F90-7285, F90-6569, F90-5058, F90-7423, LJ cultivar Vernal, and conventional cultivars Deltapine 105 (DPL-105; MG V), Bryan (MG-VI), and Braxton (MG VII) were selected for analysis.

Results and discussion: At the mid-April planting, average days to first flower (R1) for LJ genotypes was 82 d (data not shown). This period of vegetative growth was 12 d longer than DPL-105, 6 d longer than Bryan, and 4 d longer than Braxton. Flower notes were not taken at the late May planting date. Average days to full maturity (R8) for the LJ genotypes were 174 d at the mid-April planting and 141 d at the late May planting. The average LJ lifecycle was 8 and 9 d more at the mid-April and late May planting dates than DPL-105, 10 and 6 d less than Bryan, and 12 and 7 d less than Braxton, respectively.

Average LJ main stem length (103 cm) was similar to Braxton at the mid-April planting and 19 and 14 cm longer than DPL-105 and Bryan, respectively (Table 1). Branch development was more extensive for the LJ types than the conventional types at the mid-April planting as indicated by greater total branch length and number of branches. At the late May planting, the conventional types exhibited a greater reduction in main stem length than did the LJ types. Branch development, however, was somewhat similar among all entries at the late May planting and no clear contrasts could be made between the LJ and conventional types.

Trends observed for the yield component parameters corresponded with the observations for morphology in regards to enhanced LJ branch development at the early planting. At the mid-April planting, the LJ types carried a greater proportion of seed yield

did the LJ types. Branch development, however, was somewhat similar among all entries at the late May planting and no clear contrasts could be made between the LJ and conventional types.

Trends observed for the yield component parameters corresponded with the observations for morphology in regards to enhanced LJ branch development at the early planting. At the mid-April planting, the LJ types carried a greater proportion of seed yield on branches compared to the conventional types (Table 2). Yield component distribution at the late May planting was somewhat similar among all entries and no clear contrasts between LJ and conventional types could be observed.

When the planting dates were compared, LJ genotypes had greater levels of branch development at the mid-April planting compared to the late May planting (Table 3). As planting date was extended to late May, LJ main stem length decreased 28%, total branch length 54%, number of branches 51%, and total branch yield 36%. Although LJ branch seed yield decreased at the later planting, a 50% compensatory increase in main stem yield was observed.

Conclusion: Morphological and yield component characteristics of LJ genotypes vary between early and conventional planting dates in South Carolina. At mid-April plantings, LJ genotypes exhibit more branch development and produce a greater proportion of seed yield on branches compared to conventional types. Enhanced branch growth at early LJ plantings, however, does not necessarily lead to greater seed yields (kg ha^{-1}) over later plantings (Tomkins and Shipe, unpublished data). When LJ types are planted in late May, branch development decreases, branch seed yield decreases, main stem seed yield increases, and morphological and yield component characteristics are similar to conventional genotypes.

The results from this study are preliminary and the conclusions are speculative. However, previous studies using a smaller number of LJ test entries (Board, 1985; Parvez and Gardner, 1987) support the growth and developmental trends observed between planting dates for LJ gentoypes in this study.

Table 1. Influence of early and conventional planting dates on soybean morphology.

	Morphological parameter†					
	MSL		TBL		Branches	
Identity	Mid-April‡	Late May	Mid-April	Late May	Mid-April	Late May
	cm				No. plant^{-1}	
F90-5614	99	71	187	95	4.7	2.8
F90-6908	94	61	186	115	5.4	3.2
F90-6293	95	75	168	86	5.7	2.9
F90-7285	102	73	245	159	7.2	4.9
F90-6569	104	78	214	124	6.8	4.0
F90-5058	126	87	247	92	6.7	2.8
F90-7423	90	64	207	109	7.8	3.2
Vernal	117	86	396	80	9.6	2.9
DPL-105	81	61	121	92	3.5	2.9
Bryan	86	63	121	95	2.9	3.3
Braxton	101	68	142	127	4.5	5.1
LSD (0.05)	8	6	66	39	1.7	1.1

† Main stem length (MSL) and total branch length (TBL).

‡ Early (mid-April) and conventional (late May) planting dates.

Table 2. Influence of early and conventional planting dates on soybean seed yield components.

	Main stem seed yield		Total branch seed yield	
Identity	Mid-April†	Late May†	Mid-April	Late May
	g plant^{-1}			
F90-5614	8.8	15.0	10.3	11.3
F90-6908	10.9	11.7	9.1	11.0
F90-6293	13.3	17.7	16.4	8.6
F90-7285	9.7	13.4	20.5	16.3
F90-6569	7.4	12.1	18.9	11.3
F90-5058	9.8	17.3	21.3	10.1
F90-7423	7.7	13.9	19.6	10.8
Vernal	9.4	14.3	19.6	6.8
DPL-105	6.5	12.2	10.0	11.4
Bryan	8.3	11.6	3.1	10.0
Braxton	6.8	12.3	5.3	10.4
LSD (0.05)	3.0	2.4	5.0	3.7

† Early (mid-April) and conventional (late May) planting dates.

Table 3. Comparison of long-juvenile soybean morphological and yield component variables between planting dates.

Variable	Mid-April†	Late May†	LSD (0.05)
Main stem length (cm)	103	74	3
Total branch length (cm)	231	107	21
Branches (No. plant^{-1})	6.7	3.3	0.5
Main stem seed yield (g plant^{-1})	9.6	14.4	1.0
Total branch seed yield (g plant^{-1})	16.9	10.8	1.7

† Early (mid-April) and conventional (late May) planting dates.
Analysis excludes conventional cultivars used as checks.

References

Board, J. E. 1985. Yield components associated with soybean yield reductions at nonoptimal planting dates. Agron. J. 77:135-140.

Board, J. E., W. Hall. 1984. Premature flowering in soybean yield reductions at nonoptimal planting dates as influenced by temperature and photoperiod. Agron. J. 76:700-704.

Board, J. E., J. R. Settimi. 1986. Photoperiod effect before and after flowering on branch development in determinate soybean. Agron. J. 78:995-1002.

Hartwig, E. E., R. A. S. Kiihl. 1979. Identification and utilization of a delayed flowering character in soybeans for short-day conditions. Field Crops Res. 2:145-151.

Parvez, A. Q., F. P. Gardner. 1987. Daylength and sowing data response of soybean lines with "juvenile" trait. Crop Sci. 27:305-310.

Sinclair, T. R., K. Hinson. 1992. Soybean flowering in response to the juvenile trait. Crop Sci. 32:1242-1248.

Jeff P. Tomkins
Emerson R. Shipe
Kuell Hinson-USDA/ARS

UNIVERSITY OF TENNESSEE
Plant Molecular Genetics
College of Agricultural Sciences and Natural Resources
and Center for Legume Research
Knoxville, TN 37901-1071

245

Enhanced detection of Polymorphic DNA: identification of markers tightly linked to the supernodulation locus in soybean by multiple arbitrary amplicon profiling of endonuclease digested DNA

Eukaryotic or prokaryotic organisms can be identified at the DNA level using molecular hybridization or DNA amplification. These techniques detect DNA polymorphisms with which to construct linkage maps and distinguish organisms at the molecular level. Restriction fragment length polymorphisms (RFLPs) result from changes in endonuclease target sites and have been widely used as molecular markers in the construction of linkage maps in a variety of organisms, including a number of important plant species. Amplification fragment length polymorphisms (AFLPs) generated by enzymatic amplification with one or more arbitrary oligonucleotide primers (Williams et al., 1990; Welsh and McClelland, 1990; Caetano-Anollés et al., 1991a) arise from variation in number and length of amplicons arbitrarily selected for amplification. These AFLPs have been used to construct genomic maps using recombinant inbreds (Reiter et al., 1992) or as markers closely linked to specific loci by use of near isogenic lines (Martin et al., 1991) or bulked segregant analysis (Michelmore et al., 1991; Giovannoni et al., 1991).

One of these multiple arbitrary amplicon profiling (MAAP) techniques, DNA amplification fingerprinting (DAF), uses very short (≥ 5 nt) arbitrary oligonucleotide primers to direct amplification of discrete portions of a genome producing characteristic and relatively complex profiles (Caetano-Anollés et al., 1991a). Here we show that detection of polymorphic DNA can be significantly enhanced when MAAP is coupled with restriction endonuclease cleavage. The differential destruction of amplicons during cleavage enriches for polymorphisms

by "silencing" common products. Using this approach we distinguished closely related organisms and near-isogenic lines, and detected amplification fragment length polymorphisms (AFLPs) diagnostic of subtle genome alterations.

Materials and methods: Plant DNA was isolated using the method of Dellaporta et al., (1983), fungal DNA using a small-scale CsCl purification method (Yoon et al., 1991) and bacterial DNA using a phenol-chloroform extraction procedure (Bassam et al., 1992b). Glycine max (L.) Merrill lines nts382 and nts1007 (Carroll et al. 1985a,b) were crossed to Glycine soja Sieb. & Succ. accession PI468.397 under field conditions in the summer of 1988 (Landau-Ellis et al., 1991). The symbiotic mutants were used as the females, and F_1 plants confirmed as true hyubrids. F_1 and F_2 progeny were grown in the greenhouse, inoculated with Bradyrhizobium japonicum strain USDA110, and selected for the supernodulating trait. The original F_2 populations consisted of 82 (C16 series) and 57 (A3 series) plants for crosses with nts382 and nts1007, respectively. Purified DNA from F_2 supernodulating plants was analyzed by amplification.

Amplification was done in a total volume of 10 μl or 25 μl containing 3 μM heptamer or octamer or 30 μM pentamer primers, 0.3/μl of Thermus aquaticus AmpliTaq Stoffel fragment DNA polymerase (Perkin-Elmer/Cetus, Norwalk, CT), 200 μM of each dNTP (Pharmacia LKB Biotechnology Inc., Piscataway, NJ), 1.5 mM or 6 mM $MgCl_2$, 10 mM KCl, 10 mM Tris-HCl (pH 8.3), and about 1 or 0.04 ng/μl of template DNA from low and high complexity genomes, respectively. The mixture was overlaid with 2 drops of mineral oil and amplified in a dual-block thermocycler (Ericomp Inc., San Diego, CA) connected to a refrigerated water bath or in an oven thermocycler (Bios, New Haven, CT) for 35 two-step cycles of 20 sec at 96°C and 20 sec at 30°C or 1 sec at 96°C and 1 min at 55°C for amplification with heptamer and octamer primers or pentamers, respectively. Sample temperature was continuously monitored with a thermal probe. Oligonucleotide primers synthesized with >99% efficiency (National Biosciences, Annapolis, MN) were used unpurified and behaved consistently from batch to batch.

Restriction endonuclease digestions of template DNA were done directly in the amplification tube or digested prior to amplification in 10 μl volume with 2 units of enzyme according to manufacturer directions. Complete digestion was confirmed in agarose gel electrophoresis. Digestion of amplification products was in a total volume of 4 μl containing 2 μl of undiluted amplification products and 4 units of enzyme in 2 μl of 1x recommended restriction buffer. None of the recognition sequences from the restriction enzymes used were related to sequences complementary to that of the primer. Both blunt-end or staggered cutting enzymes were used.

Amplification products were separated in urea-polycrylamide gels (Caetano-Anollés et al., 1991a) and stained with silver (Bassam et al., 1991). Wells were loaded with 3 μl of a 1/10 dilution of each amplification reaction mixed with 3 μl of loading buffer (10M urea and 0.01% xylene cyanol FF). Gels backed on GelBond PAG polyester sheets (FMC, Rockland, ME) were preserved by drying.

Results and discussion: Closely related isolates of the fungal pathogen Discula destructiva, and banana (Musa sp.), soybean, and centipedegrass (Eremochloa ophiuroides) cultivars could be readily distinguished by endonuclease cleavage of template DNA or amplification products. To illustrate the power of this approach we differentiated two near-isogenic lines of soybean generated by ethyl methane sulfonate (EMS) mutagenesis. These legume mutants form profuse nitrogen-fixing nodules in the presence of Bradyrhizobium japonicum and otherwise inhibitory levels of nitrate (Carroll et al., 1985a,b). Extensive studies at the physiological level showed that supernodulation is conditioned by a shoot factor that suppresses development of Bradyrhizobium-induced infections (Caetano-Anollés and Gresshoff, 1991). The nts segregates as a single recessive Mendelian locus (Delves et al., 1988). Supernodulating allelic mutants were distinguished from each other and from their parent by simultaneous digestion with two or more restriction enzymes prior to amplification. Polymorphisms were further studied for their co-inheritance in supernodulating F_2 populations from crosses between the

mutants and G. soja. The nts trait segregates as a single recessive Mendelian locus that is tightly linked to RFLP marker pA-132 (Landau-Ellis et al., 1991; Landau-Ellis and Gresshoff, 1992). Amplification with 25 octamer and decamer primers (470 products) did not produce any AFLP in the absence of digestion. In contrast, 19 octamers and multiple digestion detected 42 AFLPs, of which 18 appeared tightly linked to the nts locus and several others lossely linked (Table 1). While many AFLPs showed Mendelian inheritance, studies in the absence of digestion show that AFLPs are not always inherited in F_1 populations from nts382 x G. soja crosses (R. Prabhu and B. Tamot, unpublished). From 67 AFLPs amplified using 24 primers, seven disappeared in the F_1 progeny apparently not originating from cytoplasmic DNA or amplification competition phenomena. New bands also appeared in the F_1 progeny.

Our results suggest that EMS is capable of inducing extensive DNA alternations that can be used to study specific genomic regions. Template endonuclease cleaved MAAP (tec-MAAP) markers could efficiently identify markers linked to a gene of interest. These markers can identify genes directly or allow their subsequent capture by cloned DNA fragments; provide anchor sites for chromosome walking; enhance bulk segregant analysis; help distinguish closely related organisms, individuals or cell lines; and become diagnostic tools in breeding programs.

Table 1. MAAP markers and co-inheritance with the nts locus.

AFLPa[a]	F_2 segregation(%)[b]	Number of AFLPs[c]
linked	100	18
loosly linked	60-87	3
unlinked	0-40	5
other	-	16

[a] Fingerprints fell into 12 categories according to the presence or absence of an amplification product in a sequence of DAF profiles defined by the order: G. soja, G. max cv. Bragg, nts382, nts1007 and F_2 segregant. Only patterns containing AFLPs between Bragg and nts mutant were scored.

[b] Co-inheritance studies in a supernodulating F_2 population from crosses between G. soja and nts1007 (15 indiniduals) and/or nts382 (20 individuals). Tight linkage occurs when the marker is co-inherited in all supernodulating F^2 progeny.

[c] Number of polymorphisms found after screening 19 octamer primers.

Genetic polymorphisms provide a powerful tool for organismal identification--parentage determination, pedigree analysis, varietal identification, cultivar certification, and monitoring of loci in breeding programs are only some examples. DAF fingerprints can be tailored to vary in the number of both polymorphic and monomorphic bands (Caetano-Anollés et al., 1991b; Bassam et al., 1992a), usually by changing primer sequence and length, annealing temperature (Caetano-Anollés et al., 1992), the number of primers used in the reaction (Caetano-Anollés et al., 1991a) and the thermostable DNA polymerase (Bassam et al., 1992b). Here we provide another way to tailor fingerprint pattern, where enzymatic digestion of template DNA or amplification products is used to increase the information content (ie. number of bands and

polymorphisms) of fingerprints generated with a particular primer.

This work was supported through the Racheff Chair of Excellence in Plant Molecular Genetics, the USB (American Soybean Association), the Tennessee Soybean Promotion Board, the Human Frontier Science Program and in part by a grant from Pioneer Hi-Bred International.

References

Bassam, B. J., G. Caetano-Anollés, P. M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 80:81-84.

Bassam, B. J., G. Caetano-Anollés, P. M. Gresshoff. 1992a. DNA amplification fingerprinting and its potential for genome analysis. In: Gresshoff P. M. (ed.) Current Topics in Plant Molecular Biology: Plant Biotechnology and Development. CRC Press, Boca Raton, pp 1-9.

Bassam, B. J., G. Caetano-Anollés, P. M. Gresshoff. 1992b. DNA amplification fingerprinting of bacteria. Appl. Microbiol. Biotech. 38:70-76.

Caetano-Anollés, G., P. M. Gresshoff. 1991. Plant genetic control of nodulation in legumes. Annu. Rev. Microbiol. 45:345-382.

Caetano-Anollés, G., B. J. Bassam, P. M. Gresshoff. 1991a. DNA fingerprinting using very short arbitrary olingonulceotide primers. Bio/Technology 9:553-557.

Caetano-Anollés, G., B. J. Bassam, P. M. Gresshoff. 1991b. DNA amplification fingerprinting: a strategy for genome analysis. Plant Mol. Biol. Rep. 9:292-305.

Caetano-Anollés, G., B. J. Bassam, P. M. Gresshoff. 1992. Primer-template interactions during DNA amplification fingerprinting with arbitrary olingonucleotides. Mol. Gen. Genet. 235:157-165.

Carroll, B. J., D. L. McNeil, P. M. Gresshoff. 1985a. A supernodulation and nitrate-tolerant symbiotic (nts) soybean mutant. Plant Physiol. 78:34-40.

Carroll, B. J., D. L. McNeil, P. M. Gresshoff. 1985b. Isolation and properties of soybean mutants which nodulate in the presence of high nitrate concentrations. Proc. Natl. Acad. Sci. USA 82:4162-4166.

Dellaporta, S. L., J. Wood, J. B. Hicks. 1983. A plant minipreparation: version II. Plant Mol. Biol. Rep. 1:19-21.

Delves, A. C., B. J. Carroll, P. M. Gresshoff. 1988. Genetic analysis and complementation studies on a number of mutant supernodulating soybean lines. J. Genet. 67:1-8.

Giovannoni, J. J., R. A. Wing, M. W. Ganal, S. D. Tanksley. 1991. Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucl. Acids Res. 19:6553-6558.

Landau-Ellis, D., S. Angermüller, R. Schoemaker, P. M. Gresshoff. 1991. The genetic locus controlling supernodulation in soybean (Glycine max L.) co-segregates tightly with a cloned molecular marker. Mol. Gen. Genet. 228:221-226.

Landau-Ellis, D., P. M. Gresshoff. 1992. Supernodulating soybean mutant alleles, nts382 and nts1007, show no recombination with the same restriction fragment length polymorphism marker. Mol. Plant-Microbe Interact. 5:572-576.

Martin, G. B., J. G. K. Williams, S. D. Tanksley. 1991. Rapid identification of markers linked to a Pseudomonas resistance gene in tomato by using random primers and near-isogenic lines. Proc. Natl. Acad. Sci. USA 88:2336-2340.

Michelmore, R. W., I. Paran, R. V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA 88:9828-9832.

Reiter, R. S., J. G. K. Williams, K. A. Feldmann, J. A. Rafalski, S. V. Tingey, P. A. Scolnik. 1992. Global and local genome mapping in Arabidopsis thaliana by using recombinant inbreds and random amplified polymorphic DNAs. Proc. Natl. Acad. Sci. USA 89:1477-1481.

Welsh, J., M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res. 18:7213-7218.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18:6531-6535.

Yoon, C., D. A. Glawe, P. D. Shaw. 1991. A method for rapid small-scale preparation of fungal DNA. Mycologia 83:835-838.

Gustavo Caetano-Anollés
Brant J. Bassam
Peter M. Gresshoff

UNIVERSITY OF TENNESSEE
Plant Molecular Genetics and Center for Legume Research
Knoxville, TN 37901

245

Soybean genes involved in cell division and possibly nodulation: cdc2 and cyclin B

The molecular mechanisms that regulate cell division have a fundamental role in the initiation of nodule meristems. The genetic and biochemical control that regulates eukaryotic cell cycle has been studied mainly in yeast and mammalian cells and so far few reports dealing with cell-cycle genes in higher plants have been published (for review, see Jacobs, 1992). The eukaryotic cell cycle is highly conserved and is regulated by a complex known as maturation promoting factor (MPF), which consists of $p34^{cdc2}$ protein kinase and cyclin (Nurse, 1990).

We plan to use the promotor region of cell-cycle genes with chimeric reporter genes to analyze their expression during early nodulation of soybean and some of its supernodulating and non-nodulating mutants (Caetano-Anollés and Gresshoff, 1991). In this report we describe the isolation and characterization of soybean genomic clones for cdc2 and cyclin, and present the patterns of their expression analyzed by Northern hybridization.

Materials and methods:

Plant material and strains: Glycine max (L.) cv. Bragg and its supernodulating nts382 and non-nodulating nod139 mutants (Carroll et al., 1985, 1986) were used in these studies. Soybean seedlings were inoculated with Bradyrhizobium japonicum USDA110, two days after sowing.

Screening of genomic library: The GEM11 phage library (Kolchinsky et al., 1993), containing 10^6 recombinants was transferred to Plaque Lift Membranes (Bio-Rad) according to manufacturer's procedures. The 960 bp cyclin cNDA (Hata et al., 1991) and 1200 bp cdc2 S6 cDNA (Miao et al., 1993) were used as probes.

Restriction mapping: In order to map genomic clones for both cyclin and cdc2, the phage DNA was partially digested with the restriction enzymes used for mapping, run in 0.6% agarose gels, transferred onto Zeta Probe membranes (Bio-Rad) and hybridized to right-terminal HindIII fragment of vector lambda GEM11. In parallel experiments the products of complete insert digestions were subjected to Southern hybridization using respective cDNA as probes.

Isolation of RNA and Northern hybridization: Total RNA from soybean tissue was isolated (Chirgwin et al., 1979) and 20 μg were fractionated on 1% agarose gels containing 2.2 M formaldehyde. Northern hybridization was performed according to the method of Gowri et al. (1991).

DNA sequence analysis: DNA was sequenced by the dideoxy chain-termination method and Sequenase Version 2.0 kit (United States Biochemicals).

Results and discussion: The genomic clones homologous to cyclin and cdc2 were isolated using GEM11 genomic library, made of cv. Bragg size-fractionated DNA. Among the 10^6 screened recombinants three positive cyclin and five cdc2 clones were isolated. The restriction maps of respective clones are shown on Fig. 1. Among the cdc2 clones two classes of genes were detected. Class 1, but not class 2, has an internal XhoI site similar to the soybean cDNA clone used for screening the library. The detection of two distinct soybean genomic clones for cdc2 confirmed the data published by Miao et al. (1993). They described two soybean cDNA clones and showed that they are differentially regulated during plant development.

Three cyclin clones were partially overlapping, thus only one clone was chosen for further characterization. The cDNA fragment was located on the map close to the left arm of the lambda vector (Fig. 1b). The 5' region of the cyclin gene was subcloned and the first 400 bp were sequenced (Fig. 2). The analyzed region shows a 51 bp deletion, which includes an ATG initiator codon, compared to the only soybean cyclin cDNA sequence described so far (Hata et al., 1991). Two other possible translational start sites were detected as well as TATA-and CAAT-like motifs. The cell cycle box (CCB) sequence AAAGCA was

found at the -139 (or -177) position with respect to the presumptive ATG codons. Although the CCB consensus sequence is CACGAAAA (Nasmyth, 1985), different variants of the CCB motif were found in cyclin genes of budding yeast. It was also shown that they are functional in reverse orientation (Ogas et al., 1991).

The expression of cyclin and cdc2 during nodule development was analysed by Northern hybridization using RNA isolated from roots of soybean cv. Bragg as well as supernodulating (nts382) and non-nodulating (nod139) mutants. The mRNA coding for cyclin (1600 bp) and $p34^{cdc2}$ kinase (1400 bp) were detected in both inoculated and uninoculated roots of cv. Bragg, nts382 and nod139 in the first analyzed stage (24h after inoculation). In Bragg and nts382 the transcript level was higher in infected roots, whereas the level in nod139 roots was similar to uninoculated plants. The expression of both cyclin and cdc2 was higher in inoculated roots of nts382 compared to cv. Bragg. The mRNA for cyclin was also analyzed in different tissues of 14 day old inoculated soybean plants. A higher transcript level was detected in root and shoot meristems than in the lateral root region. A relatively high cyclin level was found in the zone located below the emergence of lateral roots. In expanded leaves and hypocotyl, cyclin mRNA was almost undetectable. This confirms that cyclin expression was correlated with proliferating tissues.

References:

Caetano-Anollés, G., P.M. Gresshoff. 1991. Plant genetic control of nodulation. Ann. Rev. Microbiol. 45:345-382.

Carroll, B. J., D.J. McNeil, P.M. Gresshoff. 1985. A supernodulation and nitrate tolerant symbiotic (nts) soybean mutant. Plant Physiol. 78:34-40.

Carroll, B. J., D.L. McNeil, P.M. Gresshoff. 1986. Mutagenesis of soybean (Glycine max (L.) Merr.) and isolation of non-nodulating mutants. Plant Sci. 47:109-115.

Chirgwin, M. M., A. E. Przbyla, R. J. MacDonald, W. J. Rutter. 1979. Ioslation of biologically active ribonucleic acid from sources enriched in ribonucleases. Biochemistry 18:5294-5399.

Gowri, G., R.C. Bugos, W. H. Campbeli, C. A. Maxwell, R. A. Dixon. 1991. Stress responses in alfalfa (Medicago sativa L.). Plant Physiol. 97:7-14.

Hata, S., H. Kouchi, I. Suzuka, T. Ishii. 1991. Isolation and characterization of cDNA clones for plant cyclins. EMBO 10:2681-2688.

Jacobs, T. 1992. Control of cell cycle. Dev. Biol. 153:1-15.
Kolchinsky, A., E. Landau-Ellis, S. Angermuller, J. Deckert, P. M. Gresshoff. 1993. Molecular analysis of polymorphic DNA region tightly linked to supernodulation (nts) locus of soybean. The Plant Journal (in review).
Miao, G-H., Z. Hong, D. P. S. Verma. 1993. Two functional soybean genes encoding p34^{cdc2} protein kinases are regulated by different plant developmental pathways. Proc. Natl. Acad. Sci. (USA) in press.
Nasmyth, K. 1985. A repetitive DNA sequence that confers cell-cycle START (CDC28)-dependent transcription of the HO gene in yeast. Cell 42:225-235.
Nurse, P. 1990. Universal control mechanism regulating onset of M-phase. Nature 344:503-507.
Ogas, J., B. J. Andrews, Herskowitz. 1991. Transcriptional activation of CLN1, CLN2 and putative new G1 cyclin (HCS26) by SW14, a positive regulator of G1-specific transcription. Cell 66:1015-1026.

J. Deckert
N. Taranenko
P. M. Gresshoff

TCTGGGGGTC TCTCTCCCCA CCTTCTCAGA AAA----GGA AGGGGAGAAT GCAGTTGAGA
AGAGAGAGAG AGAGAT---- AGAGAGAGGG GGGGGTTCTT CTACACAGAT TTGTTTGATT
TCAATTCAAG ACCAAAGAAG AATGCACTAG TA-------- TAGGGGGAGG AAAACAACAG
AAGAAGAACG GTGTTGCTGA TGGAAGGAAC CGCAAAGCAT TGGGTGACAT TGGGAATTTG
GCCAATGTAA GAGGCGTTGT TGATGCCAAA CCAAATCGCC CCATCACAAG GAGTTTTGGT
GCACAATTAC TTGCCATGCA CAAGCAGCAG CAGCTCGTGA TAATAGCAAG AGACAAGCAT
GTGCTATGTG GCTGGTCCTC CTGCCGTTGC TAATGAAGGA GAGTTGCGGT GGCCAAAAGA

Figure 2. The nucleotide sequence of 5' region of cyclin genomic clone.
The TATA- and CAAT- like motif and CCB sequences are underlined. The presumptive ATG codons of the gene is double underlined.

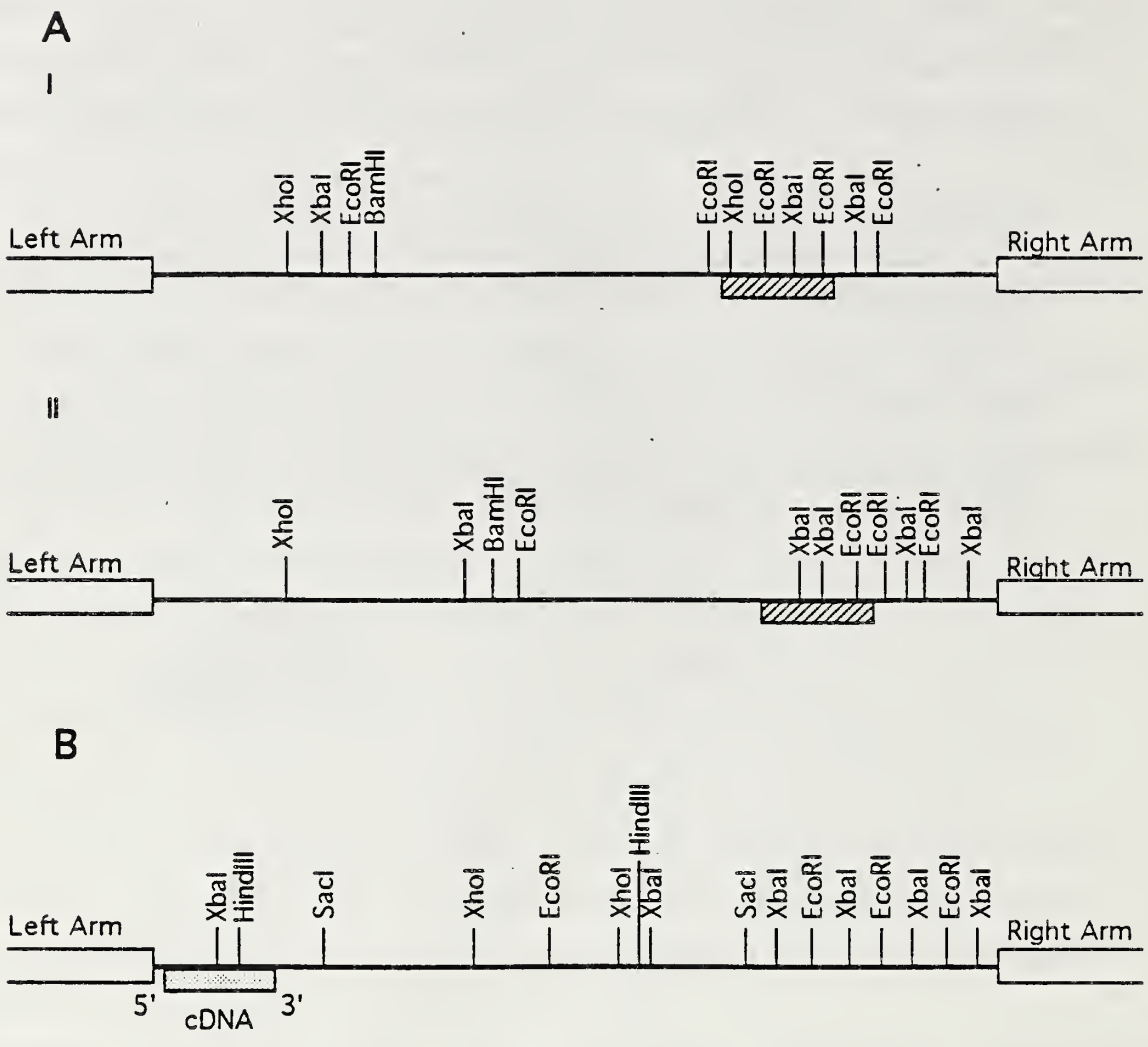


Figure 1. Restriction maps of genomic clones for cdc2 (A) and cyclin (B). Hatched boxes on cdc2 maps show putative regions of homology to the cDNA clone. Dotted box on cyclin map indicates the position of cDNA clone.

UNIVERSITY OF TENNESSEE
Plant Molecular Genetics
P.O. Box 1071
Knoxville, TN 37901-1071

241

Use of two dimensional electrophoresis to assign copies of soybean RFLP probes to large DNA fragments resolved by pulsed-field gel electrophoresis

In preparation for physical mapping and a possible chromosome walk near the nts locus of soybean (Landau-Ellis et al:, 1991; Carroll et al., 1985; Fig. 1), we developed procedures for isolating high molecular weight DNA and analyzing it by pulsed-field gel electrophoresis (PFGE; Schwartz and Cantor, 1984; Honeycutt et al., 1992; Funke et al., 1993). One objective was to correlate physical and genetic distances in a region of the soybean genome by demonstrating comigration of molecular markers on large DNA fragments. A problem we encountered was how to relate high molecular weight hybridizing bands on pulsed-field gels to the monomorphic and polymorphic copies of probes revealed by RFLP analysis. This problem was solved by applying a second dimension electrophoresis following PFGE (Fig. 2; Walter and Cox, 1992; Funke et al., 1993).

We present data for two markers, pA-36 and pA-89, located in a cluster in linkage group E of the USDA-ARS/ISU soybean RFLP map (Fig. 1; Keim et al., 1989). This cluster was chosen because the markers are close enough together that we hoped to be able to span the recombination distance between them on large DNA fragments. Three markers in this cluster have two copies in the genome, and one, pK-9, has four. Duplicated sequences probably reflect the ancient tetraploidy of soybean and are an indication of the presence of homoeologous regions in the genome (Hadley and Hymowitz, 1973; Shoemaker et al., 1992.)

Because the genetic lines of Glycine max and G. soja in use in our laboratory (cv. Bragg and PI468.397) are different from the ones that were used to construct the ARS/ISU map (A81-356022 and PI468.916), probes do not always show the expected polymorphisms in our genetic lines (probes were

kindly supplied by Randy Shoemaker, ARS/ISU, and represent PstI fragments that are polymorphic between A81-356022 and PI468.916). For example, copies of pA-89 (designated a and b) were originally mapped to two linkage groups using DraI (Fig. 1), but this enzyme reveals no polymorphism between our genetic lines (Fig. 3a). We assume that gene order and chromosomal location of markers is conserved (Shoemaker et al., 1992). Because the polymorphic copy of pA-36 (Fig. 3a) was found to be linked to a single copy marker pUTG-132a on linkage group E, we were able to use it as an anchor during physical mapping of the cluster (Landau-Ellis et al., 1992; Kolchinsky et al., 1993).

On Southern blots of pulsed-field gels, reproduced schematically in Fig. 3b, pA-89 and pA-36 appear to cohybridize on a 1000 kb NotI fragment, shortened to 970 kb by SfiI in double digests, and a 350 kb SfiI fragment. It was necessary to determine which of these fragments is colinear with the cluster of markers in linkage group E and distinguish them from fragments from the chromosomal region around pA-89b, in linkage group I. To do this we carried out second dimension electrophoresis to see which fragments contained the polymorphic copy of pA-36. The results are shown in Fig. 3c.

Both copies of pA-36 are present in the unresolved high molecular weight region (the compression zone) of pulsed-field gels, possibly indicating some methylation of rare-cutter restriction sites in the DNA surrounding them. The polymorphic copy of the marker (at 3.5 kb) is present only on the 1000 kb NotI, 970 kb NotI/SfiI, and 750 kb SfiI fragments. The unmapped monomorphic copy of pA-36 (9.0 kb) cohybridizes with pA-89 to the 350 kb SfiI band. Comigration of the polymorphic copy of pA-36 with pA-89 on 1000 kb and 970 kb fragments means that 1.8 cM of genetic distance are captured by about 1000 kb, so that there are 550 kb/cM in this part of linkage group E. We are aware that this conversion factor may not be relevant to physical mapping 16 cM away around the nts locus.

Comigration of the monomorphic copy of pA-36 with a copy of pA-89 suggests that PFGE can be used to demonstrate physical contiguity of silent or

unmapped copies of markers on the RFLP map. Finally, the presence of both copies of pA-89 in the same NotI/SfiI band, while they segregate to different linkage groups (Fig. 3c and Fig. 1), implies that this band contains two separate fragments of the same size.

In summary, this work shows that interpolating between pulsed-field gels and the RFLP map of soybean presents some problems that may be resolved by using a second dimension conventional electrophoresis.

References:

Carroll, B. J., D.L. McNeil, P.M. Gresshoff. 1985. A supernodulation of nitrate tolerant symbiotic (nts) soybean mutant. Plant Physiol 78:34-40.

Funke, R. P., A. Kolchinsky, P.M. Gresshoff. 1993. Physical mapping in a region of the soybean genome containing duplicated sequences. Plant Molecular Biology (under review).

Hadley, H. H., T. Hymowitz. 1973. In Caldwell, B. E. (ed.), Soybeans: Improvement, Production, and Uses. pp. 97-114. American Society of Agronomy, Madison, WI.

Honeycutt, R. J., B.W.S. Sobral, M. McClelland, A.G. Atherly. 1992. Analysis of large DNA from soybean (Glycine max L. Merr.) by pulsed-field gel electrophoresis. The Plant Journal 2: 133-135.

Keim, P., R.C. Shoemaker, R.G. Palmer. 1989. Restriction fragment length polymorphism diversity in soybean. Theor Appl Gen 77:786-792.

Kolchinsky, A., D. Landau-Ellis, S. Angermüller, P.M. Gresshoff. 1993. Molecular analysis of a polymorphis DNA region tightly linked to the supernodulation (nts) locus of soybean. The Plant Journal (under review).

Landau-Ellis, D., S. Angermüller, R. Shoemaker, P. M. Gresshoff. 1991. The genetic locus controlling supernodulation in soybean (Glycine max L.) co-segregates tightly with a cloned molecular marker. Mol Gen Genet 228:221-226.

Schwartz, D. C., C.R. Cantor. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell 37:67-75.

Shoemaker, R. C., R.D. Guffy, L. L. Lorenzen, J. E. Sprecht. 1992. Molecular genetic mapping of soybean: map utilization. Crop Sci 2:1091-1098.

Walter, M. A., D.W. Cox. 1992. Two-dimensional DNA Electrophoresis (2D-DE) for Mammalian DNA. In Burmeister, M. Ulanovsky, L. (eds), Methods in Molecular Biology, Vol. 12: Pulsed-field Gel Electrophoresis. pp. 285-297. The Humana Press Inc., Totowa, NJ.

R. P. Funke
A. Kolchinsky
P. M. Gresshoff

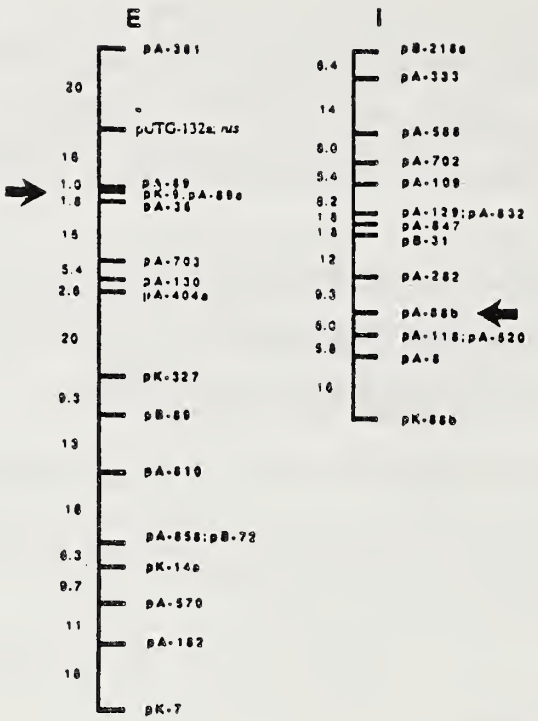


Figure 1. Linkage groups E and I of the ARS/ISU soybean RFLP map, showing the cluster of markers analyzed by PFGE.

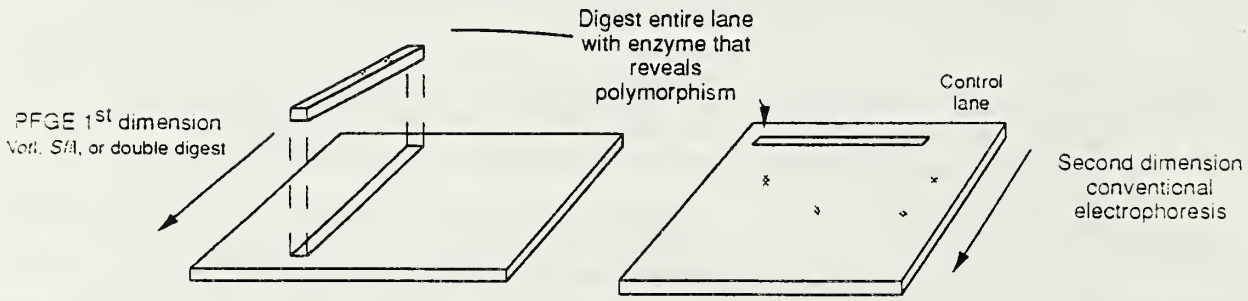


Figure 2. Two-dimensional electrophoresis for soybean DNA

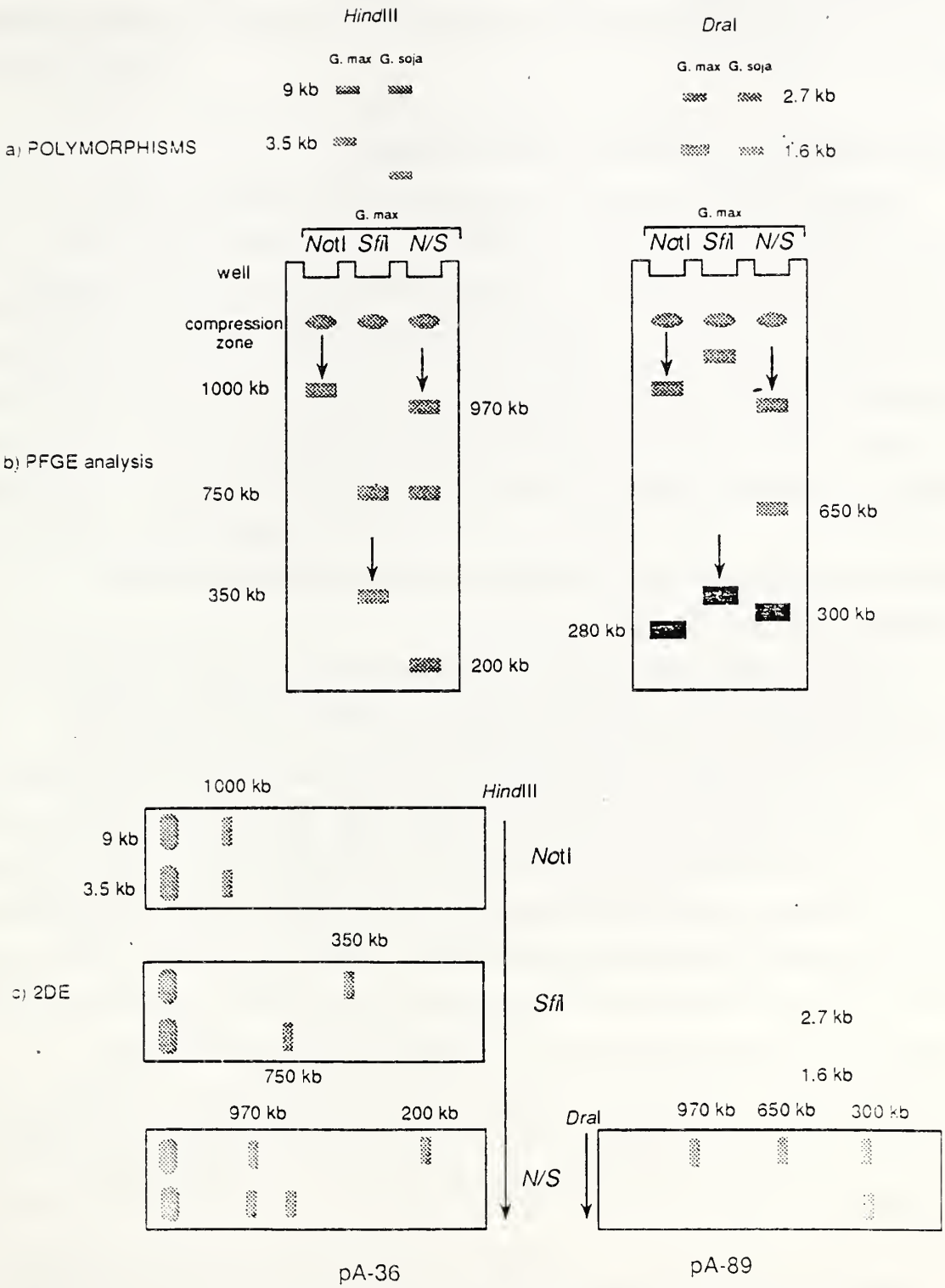


Figure 3. Schematic representation of Southern blots that are discussed in the text.

UNIVERSITY OF TENNESSEE
Plant Molecular Genetics
Knoxville, TN 37901-1071

245

Soybean telomeres as potential molecular markers

An elaborated RFLP map of the soybean genome was developed recently (ARS-USDA map) (Keim et al., 1990). It still contains slightly more linkage groups than chromosomes, and the linkage groups are not correlated with the soybean karyotype. Additional molecular markers based on the high level of polymorphism of telomeres would be very useful for further elaboration of the map. Highly polymorphic repetitive telomeric sequences were used in several mapping projects as additional markers for chromosome arms (Burr et al., 1992; Ellis et al., 1992; Ganal et al., 1992; Kleinhofs, unpublished). Here we address the question of the organization of soybean telomeres and their potential as molecular markers.

Recent studies of the organization of telomeres have shown that they usually consist of a short conserved sequence repeated 10^2-10^3 times (for review see Zakian, 1989). One strand of this sequence running in the 5'-3' direction toward the end is G- and T-rich and in most organisms does not contain C. The consensus sequence for a few plants studied is $(TTTAGGG)_n$. More proximally are usually located rather complex arrays of the subtelomeric repeats (telomere-associated sequences, TAS). This combination of highly repetitive sequences generates polymorphisms that are useful for genetic mapping of the ends of linkage groups. However, in many organisms, telomere-like sequences occur at interstitial sites of chromosomes, and as a result the molecular hybridization data produced with telomere-specific repeats are complicated and difficult to interpret (Ganal et al., 1991; Richards et al., 1991).

Since telomeres consist of long repeats of a simple 7 bp long sequence, they contain no restriction sites for most restriction enzymes. However, outside this repetitive segment a restriction enzyme that recognizes four base pairs

finds a site to cleave on average once per 300 bp. As a result, the telomeric segments appear on gels as DNA molecules much larger than average restriction products.

In order to determine the location of telomere-related sequences, a novel approach to direct labelling of the ends of chromosomes was developed (Kolchinsky and Gresshoff, 1993). It enabled us to selectively label sequences located at the ends of chromosomes. Telomeres of G. max (L.) Merr., cv. Bragg and G. soja (Sieb and Zucc., PI468.397) were labeled, DNA treated with HinfI and analysed by electrophoresis. The average size of Glycine telomeres falls roughly in between the range determined for Arabidopsis (several kilobases as shown by Richards and Ausubel, 1988) and tomato (30-60 kb, Ganal et al., 1991). There is a clear-cut polymorphism between G. max telomeres (13-18 kb) and G. soja (18-25 kb long).

To verify the telomeric nature of the labeled fragments, identical non-labeled samples were run in the same gel and hybridized to the plant telomeric probe. The probe was produced by "staggered" PCR (Ijdo et al., 1991). Two complementary 28-mers corresponding to the known sequence of A. thaliana telomeres $(TTTAGGG)_4$ (GT-primer) and $(CCCTAAA)_4$ (AC-primer) (Richards and Ausubel, 1988) were used for PCR without any additional template (1 μM primers, annealing temperature 60°C; after removal of dNTP heterogeneous products can be used for labeling). This probe revealed a picture very similar to direct labeling. Therefore, telomeric repeats in soybean chromosomes are located mostly at their ends.

The difference in the average size of telomeric regions between G. max and G. soja can be demonstrated using a number of enzymes. Moreover, even cultivars of G. max differ in the average size of their telomeres (Fig. 1). It is interesting to note that the cultivar, Peking, shares many characteristics, like seed color and nematode resistance, with G. soja.

However, direct labeling or hybridization with telomeric sequences does not reveal sufficient amount of polymorphic bands to be used for reliable mapping. Also, we used Southern hybridization with the telomeric probe to

reveal the spectrum of telomeres in segregating F2 population of the cross G. max, cv. Bragg x G. soja PI468.397. Surprisingly, the telomeres in the segregating population of F2 rearrange and non-parental bands appear (Fig. 1). Therefore, the polymorphism of the telomeric 7 bp repeats cannot be used for mapping directly.

Another source of polymorphism in the telomeric regions are TASs. They were cloned and used for this purpose in several papers. Ganal et al. (1992) used a repetitive sequence cloned earlier that proved to be located near the ends of chromosomes. Burr et al. (1992) digested total corn DNA with a 4-cutter, religated large fragments and carried out PCR with telomeric AC-primers. Kleinhofs et al. (unpublished) used ligation to vectorette construct (Riley et al., 1990) and subsequent PCR with vectorette primer and telomeric AC-primer.

We tried to run the PCR-reaction with single telomeric primers after restriction and ligation of soybean DNA according to Burr et al. (1992). Surprisingly, the PCR on control non-treated DNA with both GT and AC-primer run in stringent conditions produced sharp, reproducible and polymorphic bands shown in Fig. 2. The pattern of these bands was variety-specific, reproducible and, in a few cases studied, co-dominant. Therefore, sufficient polymorphism between parents allows one to differentiate all three possible genotypes in segregating population. These polymorphic PCR-derived bands will be mapped on the RFLP map of soybean. Besides, this simple "fingerprinting" technique can be used for variety identification because of the extensive polymorphism of the resulting pattern.

Meanwhile, we undertook cloning and sequencing of the PCR-products described above in search of potential TASs. We focused on AC-primer derived products because we assumed that there are better chances that they originate from telomeric regions. Three of those products were sequenced completely (TAS 1-1, PCR-product of G. soja DNA, 570 bp; TAS 4-1, G. max DNA, 417 bp; TAS 5-4, G. soja DNA, 568 bp) and two more partially (TAS5-6, G. soja DNA,

600 bp; TAS6-1, G. max DNA, ~1000 bp). Analysis of the obtained sequences led us to the following conclusions.

1. All sequences are highly asymmetric with clear G- and T-rich strand and C- and A-rich strand. Such asymmetry was found in other TASs sequenced so far.

2. They hybridize to large fragments of soybean DNA produced by 4-cutters. This is a common property of tandem repeats.

3. Sequence TAS4-1 contains numerous degenerated homologies to the consensus plant telomeric repeat CCCTAAA. They tend to concentrate near one end of the sequenced fragment supporting the model of telomere organization proposed by Richards et al. (1992) that postulates gradual degeneration of the 7-base repeat toward the centromere.

4. Soybean TASs have no homology to published sequences of plant TASs. Indeed, TASs are assumed to evolve fast and be under little functional constraints (Broun et al., 1992).

5. TAS1-1 contains long direct repeats revealed even at high stringency of homology search. These repeats are not related to the major 7 bp repeat. Similar pattern was found in some cloned TASs (Burr et al., 1992).

6. TAS5-4 contains a perfect palindrome and no long direct repeats. Areas of cloned TASs which have no homologies with 7 bp repeat, will be used to develop more specific PCR-test suitable for mapping of cloned sequences.

References:

Broun, P., M. W. Ganal, S. D. Tanksley. 1992. Telomeric arrays display high levels of heritable polymorphism among closely related plant varieties. Proc. Nat. Acad. Sc. USA 89:1354-1357.

Burr, B., F. A. Burr, E. C. Matz, J. Romero-Somerson. 1992. Pinning down loose ends: mapping maize telomeres and factors affecting their length. The Plant Cell 4:953-960.

Cheng, J. F., C. L. Smith, C. R. Cantor. 1989. Structural and transcriptional analysis of a human subtelomeric repeat. Nucleic Acids Res. 17:6109-6127.

Ellis, T. H. N., L. Turner, R. P. Hellens, D. Lee, T. L. Harker, C. Enard, C. Domoney, D. P. Davis. 1992. Linkage maps in pea. Genetics 130:649-663.

Ganal, M. W., N. L. V. Lapitan, S. D. Tanksley. 1991. Macrostructure of the tomato telomeres. The Plant Cell: 3:87-94.

Ganal, M. W., P. Broun, S. D. Tanksley. 1992. Genetic mapping of tandemly repeated telomeric DNA sequences in tomato. Genomics 14:444-448.

Ijdo, J. W., R. A. Wells, A. Baldini, S. T. Reeders. 1991. Improved telomere detection using a telomere repeat probe $(TTAGGG)_n$ generated by PCR. Nucleic Acids Res. 19:4780.

Keim, P., B. W. Diers, T. C. Olson, R. C. Shoemaker. 1990. RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126:735-742.

Kolchinsky, A., P. M. Gresshoff. 1993. Direct end-labeling of telomeres. Genome, in press.

Richards, E. J., F. M. Ausubel. 1988. Isolation of a higher eukaryotic telomere from Arabidopsis thaliana. Cell 53:127-136.

Richards, E. J., H. M. Goodman, F. M. Ausubel. 1991. The centromere region of Arabidopsis thaliana chromosome 1 contains telomere-similar sequence. Nucleic Acids Res. 19:3351-3357.

Richards, E. J., S. Chao, A. Vongs, J. Yang. 1992. Characterization of Arabidopsis thaliana telomeres isolated in yeast. Nucleic Acids Res. 20:4039-4046.

Riley, J., R. Butler, D. Ogilvie, R. Finniear, D. Jenner, S. Powell, R. Anand, J. C. Smith, A. F. Markham. 1990. A novel, rapid method for the isolation of terminal sequences from YAC clones. Nucleic Acids Res. 18:2887-2890.

Zakian, V. A. 1989. Structure and functions of telomeres. Annu. Rev. Genet. 23:579-605.

Alexander Kolchinsky
Peter M. Gresshoff

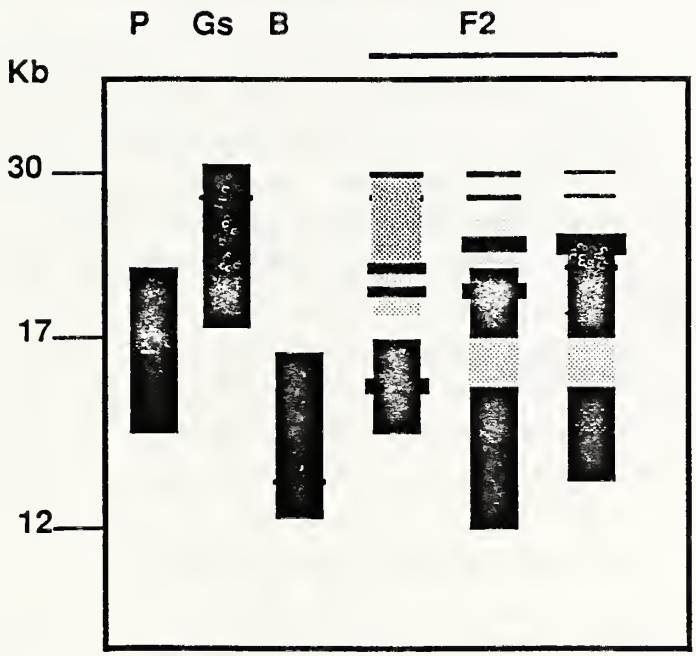


Figure 1. Polymorphism of telomeric sequences in soybean (scheme). DNA was digested with *Hin*dIII, fractionated in 0.6% agarose at 1 V/cm for 42 h and hybridized with telomeric probe described in the text. P - *G. max*, cv. Peking; Gs - *G. soja* PI468.397; B - *G. max*, cv. Bragg; F2 - three plants from segregating population *G. max* x *G. soja* (see text). Molecular sizes are indicated on the left. Computer generated *camera lucida* drawings are provided to match the reproduction ability of the Newsletter.

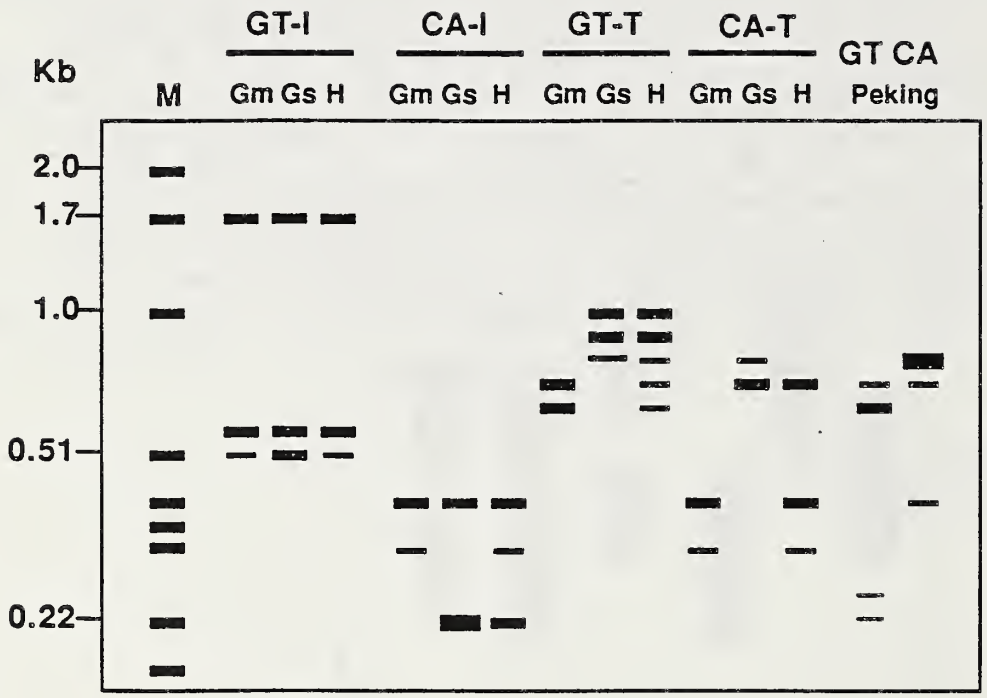


Figure 2. PCR-products generated by telomeric primers in single primer reactions (scheme). M- marker lane. GT-I - products obtained with GT-primer on DNA from *G. max* (lane Gm) and *G. soja* (lane Gs) used in the original crosses in Iowa, namely *G. max* cv. A81-356022 and *G. soja* PI468.916. H designates mixture of corresponding DNAs in equal amounts (model heterozygote). CA-I - same DNA samples, AC-primer. GT-T - GT-primer, DNA from *G. max* cv. Bragg (lane Gm) and *G. soja* PI468.397 (lane Gs) used in the original crosses in

UNIVERSITY OF UTAH
Department of Biology
Salt Lake City, UT 84112

241

1) Molecular phylogeny as a tool for soybean breeding II

Previously (Lark et al., 1992), we reported the use of Rapidly Amplified Polymorphic DNA (RAPD) markers (Tingey and del Tufo, 1993) in establishing soybean phylogeny and discussed the possible use of these phylogenies in problems relevant to soybean breeding. The data used were collected in an undergraduate laboratory (Biology 360) at the University of Utah. In a similar class this year, we have extended those studies to examine 13 perennial species of Glycine as well as a number of soybeans (*G. max*) all of which are listed in Table 1. We report here the phylogeny of the perennial species, their relation to *G. max* and the use of character distance as a possible tool for analyzing the relative genetic contribution of parents to progeny selected during a breeding program.

Materials and Methods

Seeds of perennial species were obtained from William Davis, Ring Around Seed Co. Seeds of soybean cultivars were obtained from Randy Nelson at the University of Illinois. RAPD markers were determined using the procedure described previously (see Lark et al., 1992, for materials and procedures). In brief, DNA was isolated from dried plant leaves, used as a template in PCR reactions with different 10mer primers, and the products separated on agarose gels. The presence or absence of specific size fragments were noted as character states, where each size of fragment, derived from a specific primer corresponded to a character. In all, character states were evaluated for 798 characters (fragments) produced by PCR reactions using 29 primers. Two technological improvements over the previous study were: (A) the assessment of template plant DNA concentrations used a fluorometric technique. DNA was appropriately diluted and mixed with the dye Hoechst 33258 and the quantity of DNA measured fluorometrically using a Hoefer TKO 100 mini-fluorometer; (B) Computer analysis of PCR amplified fragments, carried out by video capture of the image of ethidine bromide stained agarose gels on a Macintosh IIci computer using the program NIH image 1.42. Images were sharpened using the enhancement options in this program and analyzed by noting the coordinates of specific fragments on the gel relative to each other or to standards.

As before, phylogenies were determined by Parsimony Analysis using the program PAUP on Macintosh computers The same program was used to determine the character distances separating different taxa.

Results and Discussion

Phylogeny of the perennial glycine species

Figure 1

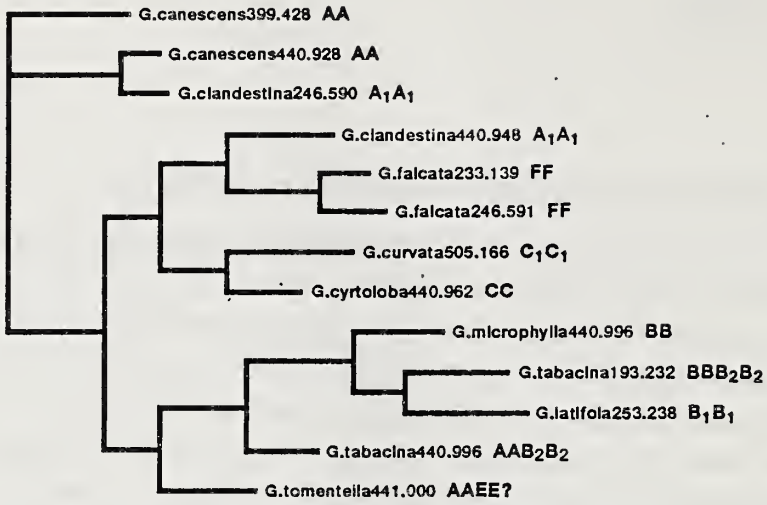


Figure 1 presents a phylogram of the 13 different perennial plants listed in Table 1. The taxonomic grouping of these species, as determined by Hymowitz (Hymowitz and Singh 1992, T. Hymowitz, personal communication) is shown to the right of each taxon. In general, our phylogeny agrees well with the results of Hymowitz and his collaborators. Three of the *G. canescens* and *G. clandestina* (Hymowitz' group A) are clustered in one clade, as are the F group, *G. falcata*, and the C group, *G. curvata* and *G. cyrtoloba*. Finally, there is a separate clade for the B group containing *G. microphylla*, *G. tabacina*, and *G. latifolia*. *G. tabacina* 410.996 is outside of this clade, closer to the A group consistent with the taxonomy of Hymowitz. The taxonomy of *G. tomentilla* 441.000 is not yet clear but its position would be consistant with a classification of AAEE.

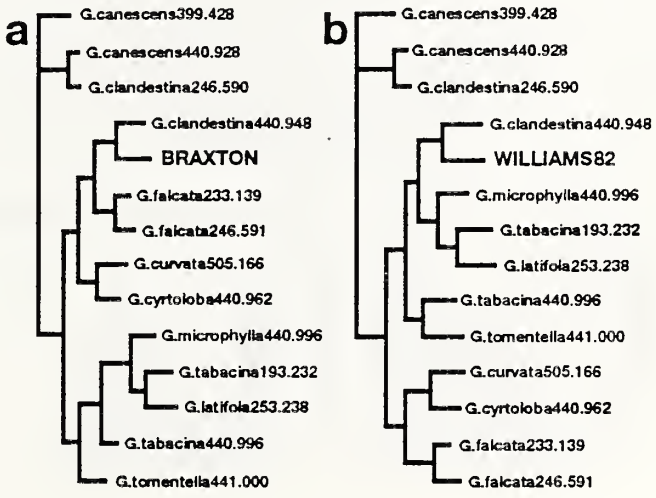
An anomaly is *G. clandestina* 440.948 which is found in a subclade with the F group. Figure 2 shows the plant from which this DNA was extracted compared to a *G. falcata* on the left and *G. clandestina* 264.590 on the right. Clearly its leaf morphology places it with the other clandestina.

Figure 2

Relationship of G. max cultivars to the perennial species

G. clandestina 440.498 is also unique in its relationship to *G. max*. We have determined the phylogenetic relatedness of each of 25 *G. max* cultivars to the perennial species in Figure 1. This was done by adding data for each soybean cultivar separately to the data for the group of perennials and constructing a phylogenetic tree using PAUP. In every case, each *G. max* was closest to *G. clandestina* 440.948. Figure 3 shows trees using Braxton or Williams 82. In the case of Braxton the soybean forms a subclade with *G. clandestina* 440.948 without any other rearrangement in the phylogenetic tree. Williams 82, however, causes rearrangements in the tree. Whereas it pairs with *G. clandestina* 440.948, parsimony can only be preserved by rearranging the tree such that the "B" clade is brought closer to the soybean. (The patristic distance between Williams 82 and the B clade is less than that between the B clade and Braxton.) This was characteristic of the other soybeans. Whereas each one paired with *G. clandestina* 440.948, their relationships to other perennial species varied, often causing different rearrangements in the perennial phylogenies in which they were included.

Figure 3



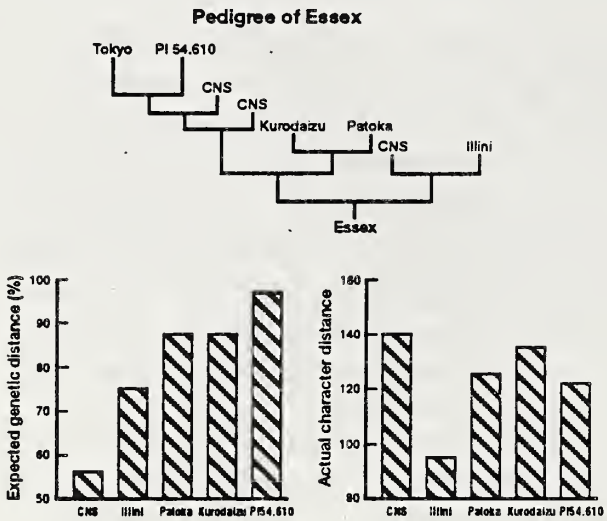
Not only is *G. clandestina* 440.948 closer to domesticated soybeans by virtue of parsimony — i.e., minimizing the number of character changes in the phylogeny; but, in almost all cases, it also is closest in terms of character distance (the number of character states [fragments] which distinguish two plants from each other). The character distances (using all 29 primers) between different plants and *G. clandestina* 440.948 are included in Table 1 where accessions have been ranked according to their distance from *G. clandestina* 440.948. Note the preponderance of *G. max* cultivars at the top of the table. An interesting finding is that CNS has the least differences for the 29 primers used in this study (i.e. is closest to this perennial). In our previous studies CNS was anomalous, often behaving as if it was closer to the perennial species than to *G. soja*. Relative values of the character distances vary with the primers used and different primers can be used to distinguish different plants suggesting that these primers reflect different portions of the genome (data not shown).

Our data should **not** be over interpreted as suggesting that *G. clandestina* 440.948 is an ancient ancestor of the modern soybean. However, they do suggest that further studies of close relatives of this taxon are merited and that it is an excellent candidate for hybridization with *G. max* in experiments of the type already carried out by Hymowitz and his coworkers. Indeed, analyses such as the ones described here could suggest which soybean (CNS?) would most likely donate a large portion of their genome to a hybrid construct.

Use of character distances to analyze cultivars produced by breeders

The analysis described above also can be used to analyze the contribution of parents to progeny selected in breeding experiments. Figure 4 shows the parentage of Essex and compares statistically expected character distances with the distances found in our analysis. In the bar graph to the left, the expected genetic distance of the genome from each parent is shown (as 100-(% of the parental genome expected)). In the histogram to the right we present the character distance (number of different character states) actually found. It can be seen that although CNS was frequently used in the

Figure 4



construct, its genome was not well retained during breeding, whereas the genome of a distant parent PI 54.610 is represented in higher than expected amounts. Similarly (results not shown) Williams 82 was found to contain much more of the Kinwa genome than would have been expected after the large number of recurrent back crosses to Williams which were part of the breeding program which produced this cultivar. It is not surprising that breeders select especially desirable progeny for their purposes and that therefore these progeny do not conform to the statistically expected proportions of parental genes. However, the type of analysis presented here can inform the breeder of the genetic basis of his result and thus provide insights useful in designing future breeding protocols.

Acknowledgement

This research was carried out in Biology 360, an undergraduate laboratory on Molecular Evolution, part of an undergraduate initiative supported by a grant from the Howard Hughes Medical Institute to the Department of Biology.

References

Hymowitz, T. and R. J. Singh, 1992. Biosystems of the Genus Glycine, 1991. Soybean Genetics Newsletter 19:184-185

Lark, K. G., J. Evans, F. Basha, R. Bogden, R. Copeland, R. Ellison, D. Horne, K. Lee, K. McDonald, J. Pierson, W. Schuster, P. Wilhelm and Y. Yu, 1992. Molecular Phylogeny as a Tool for Soybean Breeding. Soybean Genetics Newsletter 19:174-181

Tingey, S.V. and J. P. del Tufo, 1993. Genetic Analysis with Randomly Amplified Polymorphic DNA Markers. Plant Physiol. 101:349-352

K. G. Lark*
J. Evans**
P. Wilhelm**
S. Atkinson***
M. Christensen***
B. Crockett***
B. L. Dunford***
C. E. Jones***
S. Mendenhall***
T. E. Messick***
C. C. Prout***
P. E. Solomon***
M. E. Stevens***
I. Terry***
C. Walker***
C. L. Wong***
R. Ellison****

*Faculty, ** teaching assistants, ***students, ****research assistant

Table 1. Glycine accessions ranked by increasing character distance from *G. clandestina* 440.948

ACCESSION	GENOME*	CHARACTER DISTANCE
G. clandestina 440.948	A_1A_1	
CNS		210
Delmar		219
Midwest		222
Braxton		226
Tokyo		227
Minsoy		227
Richland		230
Noir		234
G. falcata 233.139	FF	235
Kurodaizu		236
Dunfield		239
Essex		241
G. cyrtoloba 440.962	CC	242
Haberlandt		244
Elf		244
Palmetto		244
PI 54610		245
Bedford		247
Mandarin		247
Williams 82		250
Lincoln		251
Biloxi		251
G. tabacina 440.996	AAB_2B_2	252
Peking		252
G. curvata 505.166		252
Manchu		253
G. clandestina 246.590	A_1A_1	254
Kingwa		255
G. falcata 246.591	FF	255
Illini		257
G. canescens 399.428	AA	257
PI 88788		261
G. tomentella 441.00	AAEE?	264
G. tabacina 193.232	BBB_2B_2	265
G. canescens 440.928	AA	267
Forrest		275
Patoka		276
G. latifolia 253.238	B_1B_1	279

*From Hymowitz and Singh, 1992, and Hymowitz personal communication.

VIRGINIA STATE UNIVERSITY
Agricultural Research Station
P.O. Box 9259
Petersburg, VA 23806

245

Fatty acid composition of soybean seeds produced in China and Virginia

Introduction: Virginia State University is currently involved in evaluation of vegetable soybean germplasm for nutritional quality (Mohamed and Rangappa, 1992a,b and Mohamed et al. 1991.) During 1990, twenty-five vegetable soybean genotypes were obtained from several agricultural research stations in China and Taiwan (Mohamed et. al. 1992a,b) to expand the germplasm collection. Since oil and protein in soybean are not only conditioned by their inheritance, but also influenced by the environmental conditions where the crop was grown (Taira and Taira, 1971; Hu et al., 1990; and Xiangxum et al., 1991), comparison of seed produced in China with that produced in Virginia might lead to a better understanding of environmental effects on fatty acid composition.

The objective of this study was to compare the fatty acid composition in original seed received from China with the seed production in Virginia. The green immature seeds of these new genotypes were also evaluated for fatty acid composition.

Materials and methods: Because of the limited number of seeds, 20 seeds from the original seed lot of each genotype were used for oil extraction and fatty acid analysis. The remaining seeds of each genotype were planted in the greenhouse under controlled environmental conditions. The seeds harvested from greenhouse grown plants were planted during 1991 in the field at Randolph farm of Virginia State University in Petersburg, VA. Immature green seeds were collected from the field planting at R_6 (Fehr and Caviness, 1977) and at maturity for fatty acid analyses. The oil was extracted and used for fatty acid analysis as described by Mohamed Rangappa (1992a).

Results and discussion: Significant variations for fatty acid content were observed among the genotypes and between original seeds and the seeds produced in Virginia (Table 1). Palmitic, oleic, linoleic and linolenic constituted 95% of the total fatty acids. The mean palmitic acid (10.73%) from Virginia seeds was significantly higher than that in seeds from China (7.50%). No significant differences were found in stearic acid ($C_{18:0}$) between Virginia and Chinese seeds, however, mean oleic acid ($C_{18:1}$) was significantly higher in the original seeds (34.01%) as compared to Virginia seeds (21.81%). In contrast, means of linoleic ($C_{18:2}$) and linolenic acids ($C_{18:3}$) in Virginia seeds were 46.28 and 7.04 percent, respectively, which were higher than those in Chinese seeds (37.36 and 5.81 percent, respectively). The Virginia seeds of all genotypes had higher $C_{22:0}$ and $C_{24:0}$ fatty acids as compared to the original seeds. G9053 had the lowest content of $C_{18:3}$ (1.09%) in both China and Virginia seeds, whereas AGS314 had the highest (10.7%). From the nutritional point of view, higher content of $C_{18:3}$ is desirable due to health benefits. Therefore, AGS314 (17.84% $C_{18:3}$) was identified as a desirable vegetable type genotype to provide immature green seeds for human consumption. It should also be noted that in case of mature seeds, the seed produced in Virginia had higher percentages of unsaturated fatty acid than the seed produced in China indicating a profound effect of environment.

The mean of total unsaturated fatty acids ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$, and $C_{20:(1-5)}$) in immature seeds was 67.91% for all fatty acids which is lower than that in mature seeds (Table 2). Oleic acid ($C_{18:1}$) had the highest concentration (28.1%) followed by linoleic acid (27.58%). The mean percent of linolenic acid ($C_{18:2}$) in immature seed (6.9%) was not different from that in mature seed (7.04%, Table 1). Percentages of palmitic and stearic were significantly higher in immature than in mature seed. The means of total unsaturated ($C_{20:(1-5)}$), $C_{22:0}$, and $C_{24:0}$ fatty acids were significantly higher in immature than in mature seeds. A wide variation for fatty acid composition was observed among gentoypes. This is in agreement with earlier reports (Mohamed and Rangappa, 1992a). AG314, AGS129, BLUESIDE, and AGS290 were identified to be

desirable genotypes for nutritional quality improvements of soybean. Additionally, it was observed that environmental effects are not similar on various fatty acids in soybean seeds. It may, therefore, be worthwhile to identify environments that positively affect the content of a desirable fatty acid.

References

Fehr, W. R., C. E. Caviness. 1977. Iowa Agri. and Econ. Exp. Stn. Special Report 80. Ames, Iowa.

Hu, Mingxiang. 1990. Soybean Sci. 9(1):41-48.

Mohamed, A., M. Rangappa. 1992a. Food Chem. 44:277-282.

Mohamed, A. M. Rangappa. 1992b. Plant Food for Human Nutr. 42:87-96.

Mohamed, A., H. Bhardwaj, M. Rangappa, and N. Pearson. 1992a. Soybean Genet. Newsl. 19:132-135.

Mohamed, A., H. Bhardwaj, M. Rangappa, N. Pearson. 1992b. Soybean Genet. Newsl. 19:136-139.

Mohamed, A., T. Mebrahtu, M. Rangappa. 1991. Plant Food for Human Nutri. 41:89-100.

Taira, Harue, H. Taira. 1971. Proc. Crop Sci. Soc. Japan 40:530-544.

Xiangxum, M., W. Shuming, L. Aiping, M. Hu. 1991. Soybean Genet. Newsl. 18:113-116.

A. Mohamed
H. Bhardwaj
M. Rangappa
N. Pearson

Table 1. Fatty acid composition of mature seed of vegetable soybean grown in China and Virginia

Accessions	Fatty acids %																			
	$C_{14:0}$		$C_{16:0}$		$C_{18:0}$		$C_{18:1}$		$C_{18:2}$		$C_{18:3}$		$C_{20:0}$		$C_{20:1}$&$_{20:4}$		$C_{22:0}$		$C_{24:0}$	
	C*	Va*	C	Va	C	Va	C	Va	C	Va	C	Va	C	Va	C	Va	C	Va	C	Va
AGS129	T**	5.84	9.88	11.7	3.79	4.58	18.5	13.6	55.3	49.1	9.6	7.28	0.29	1.21	2.09	5.5	0.56	T	T	1.2
AGS269	0.20	0.83	10.4	6.13	3.71	29.0	16.8	12.8	58.9	43.3	9.56	6.09	0.56	0.27	T	0.25	T	T	T	1.2
AGS292	T	1.58	7.08	11.4	2.39	3.44	16.4	14.5	35.1	44.2	14.2	6.49	T	T	24.8	1.11	T	T	T	1.7
AGS293	14.1	T	0.83	9.93	2.99	2.60	70.9	31.8	10.1	47.3	0.45	6.66	T	0.25	0.73	1.3	T	T	T	0.2
AGS314	13.6	3.12	5.60	9.61	0.45	5.57	64.5	15.3	15.5	50.2	0.44	10.7	T	T	T	T	T	3.88	T	1.7
G 9053	8.41	1.60	3.65	23.9	23.3	10.0	54.0	38.0	9.85	16.7	0.49	1.09	T	T	0.33	T	T	0.71	T	1.8
G10134	10.0	0.12	2.99	8.89	25.3	3.05	48.9	30.2	11.0	51.7	1.26	4.76	T	0.36	0.54	T	T	0.78	T	T
GC84136-P4-1-8	T	1.48	10.8	8.84	3.22	3.79	20.4	20.2	54.6	53.7	10.1	9.2	3.0	0.35	1.02	2.1	T	5.19	T	2.9
1AC-100	T	2.14	12.0	11.9	3.82	4.14	17.5	15.0	48.9	46.4	6.54	8.82	T	1.3	2.82	3.19	T	3.49	T	1.9
BLUESIDE	T	1.11	9.27	5.92	3.25	2.31	19.8	29.6	57.1	54.8	10.4	9.16	T	T	0.22	T	T	3.93	T	T
N2899	T	5.66	11.0	9.77	3.72	3.89	26.4	18.9	54.6	51.7	0.86	7.15	T	0.37	3.48	T	T	1.19	T	1.4
Maan	9.26	2.35	7.50	10.7	6.90	6.57	34.0	21.8	37.4	46.3	5.81	7.04	1.28	0.59	4.00	2.24	T	2.70	T	1.6

* C: Original seed from China or Taiwan, Va : Seed produced in Virginia
** T: Trace amounts

Table 2. Fatty acids composition of immature seed of vegetable soybeans grown under Virginia conditions

Accessions	oil %	Fatty acids %									
		$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$^{*}C_{20:(1-5)}$	$C_{22:0}$	$C_{24:0}$
AGS129	6.03	1.79	11.09	15.30	21.83	7.35	0.89	0.34	2.74	1.38	6.48
AGS269	5.83	0.14	4.58	2.54	24.84	25.28	4.21	T**	13.09	T	T
AGS290	6.97	0.82	19.34	5.93	24.18	11.83	29.46	0.59	6.58	T	1.28
AGS292	6.60	0.27	11.35	5.56	28.36	28.60	6.63	3.21	13.50	T	1.79.
AGS293	7.30	T	4.70	18.27	41.31	32.24	2.51	T	T	T	T
AGS314	6.51	0.3	19.26	8.29	27.41	25.02	17.84	0.55	T	1.17	T
KVS124	6.8	4.27	37.82	10.46	41.11	1.85	0.41	1.06	0.35	T	0.81
G9053	4.97	1.95	10.88	3.69	19.33	55.05	9.20	T	T	T	T
GC84136-P-4-1-8	4.53	0.56	29.23	5.92	21.29	35.88	7.12	T	T	T	T
1AC-100	4.93	2.79	12.86	4.43	19.74	50.23	11.88	0.23	T	0.36	T
BLUE SIDE	4.27	0.74	19.74	28.91	43.25	4.50	1.25	0.56	T	T	T

$^{*}C_{20:(1-5)}$: The total of different unsaturated fatty acids of C_{20}

** T: Trace amounts

Table 2 (continued). Fatty acids composition of immature seed of vegetable soybeans grown under Virginia conditions

Accessions	oil %	Fatty acids %									
		$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:(1-5)}$	$C_{22:0}$	$C_{24:0}$
HENON 33	7.0	0.09	16.39	1.63	22.31	49.35	8.75	0.44	T	T	T
HEFENG25	5.5	0.25	11.54	3.24	23.78	51.34	5.72	0.34	0.29	1.2	T
ZIHUA 4	7.4	0.89	11.28	6.01	13.58	49.53	10.64	T	T	4.06	3.56
MEIHE 3	6.3	0.4	37.15	18.00	34.56	2.79	0.15	1.13	0.41	T	1.77
N1535-1	6.5	3.41	2.89	1.93	25.97	41.14	12.57	T	T	2.89	7.49
N1831	4.0	0.31	11.16	4.20	19.59	50.32	8.24	0.46	T	1.67	T
N2899	5.3	0.59	36.69	14.38	36.06	5.11	0.57	0.96	T	5.64	T
N2962	6.4	2.21	9.95	3.71	39.89	34.97	5.22	0.32	T	T	3.71
N7788	6.3	0.46	40.39	14.96	36.41	9.76	1.2	0.38	T	T	0.47
N8806	6.5	1.06	35.93	13.86	38.06	7.09	0.98	0.37	0.55	1.46	0.65
Mean	6.0	1.22	18.77	9.11	28.11	27.58	6.93	0.73	4.69	2.20	2.80

*$C_{20:(1-5)}$: The total of different unsaturated fatty acids of C_{20}
** T: Trace amounts

UNIVERSITY OF WYOMING
Plant, Soil and Insect Sciences
Laramie, Wyoming 82071
and
Iowa State University
Department of Agronomy
Ames, IA 50011

245

Origin and stability of a new mutation for dilute-purple anthocyanin pigmentation.

A new mutation for dilute-purple anthocyanin pigmentation of flowers was identified in the 'w4-mutable' line of soybean (Glycine max [L.] Merr.) (Palmer and Groose, 1993). The dilute-purple phenotype (also referred to as 'purple-throat' and characterized by purple pigmentation in the throat of the standard petal) was originally described by Hartwig and Hinson (1962) and is exhibited by lines of genotype W1W1 W3W3 w4w4. Standard petals of the new mutant, however, exhibited a larger and more intensely pigmented purple throat than did typical dilute-purple lines. To distinguish between the new dilute-purple phenotype with the large purple throat from the previously described dilute-purple phenotype with the small purple throat we have designated them as 'dilute-purple-L' and 'dilute-purple-S,' respectively.

Genetic analyses established that the dilute-purple-L phenotype is conditioned by a new allele of the w4 locus that is recessive to the wild-type W4 allele (purple pigmentation) and dominant to a recessive w4 allele (near-white pigmentation) (Palmer and Groose, 1993). The gene symbol w4-dp was assigned to the new allele. This communication outlines the origin of the w4-dp allele and discusses stability of the allele.

The dilute-purple-L mutant was observed as a single F_{11} plant among 43 self-progeny of a pale-flowered F_{10} plant of the w4-mutable line designated 'W4M-2-8-9-2-4' where 'W4M' designates 'w4-mutable' and '2', '8', '9', '2' and '4' identify the F_6, F_7, F_8, F_9, and F_{10} parentage, respectively. (For more details regarding the pedigree of the w4-mutable line and the derivation of its sublines see: Groose et al., 1990; Palmer et al., 1990; Weigelt et al., 1990). The 43 self-progeny of this pale-flowered F_{10} plant

segregated for 27 pale-flowered plants, 15 near-white plants, and the single mutant F_{11} plant (W4M-2-8-9-2-4-22) that exhibited the new dilute-purple-L phenotype. The approximately 3 pale : 1 near-white segregation of self-progeny of W4M-2-8-9-2-4, together with other information from pedigree records, indicates that the F_{10} plant developed from a zygote heterozygous at the w4 locus for a pale allele and a recessive near-white w4 allele. This F_{10} plant descended from a mutable F_8 ancestor (W4M-2-8-9) that was used in a study of developmental timing of germinal reversion of the w4-m allele (Groose et al., 1990) and that was homozygous for mutable alleles (w4-m w4-m). Therefore, both the pale and near-white alleles carried by F_{10} plant W4M-2-8-9-2-4 were derived from the w4-m allele.

Flowers of the F_{11} plant W4M-2-8-9-2-4-22 were uniformly dilute-purple-L, and its self-progeny segregated approximately 3 dilute-purple-L : 1 near-white, indicating that the variant plant arose from a zygote that carried a novel allele of the w4 locus conditioning the dilute-purple-L phenotype and a stable recessive w4 allele. The pale allele carried by the F_{10} parent is the subject of current research and seems to be genetically unstable (RW Groose, SM Schulte, RG Palmer, unpublished results). Therefore, we suspect that the pale allele is the direct progenitor of the new w4-dp allele.

The dilute-purple-L mutation was derived from a mutable (w4-m w4-m) plant via an unstable pale flower mutation, but the w4-dp allele seems quite stable. We have not observed wild-type revertant sectors in the near-white parts of individual dilute-purple-L flowers. In two instances, however, we have seen a single, entirely purple flower borne on a plant on which all other flowers were dilute-purple-L. One example occurred in a family segregating both for dilute-purple-L and near-white flowers, which was descended from the original mutant. The second example occurred in an F_3 population homozygous for dilute-purple-L flowers. Six examples of dilute-purple-L plants with more than one mutable flower (purple sectors on a near-white background) have been observed among plants descended from the original mutant, W4M-2-8-9-2-4-22.

Possible germinal reversion of w4-dp to wild-type W4 has been noted in several instances. Five purple plants were found among plants of T321 (three instances) and

among F_2 plants from a cross between T321 and a dilute-purple-S (W3 W3 w4 w4) Harosoy isoline (two instances; see Cross 3 in Table 1 of Palmer and Groose, 1993). Self-pollinated seed from the first three plants were harvested, and the next generation segregated approximately three purple : 1 dilute-purple-L. These results would be expected if a natural outcross with a wild-type purple male parent had occurred. The other two purple-flowered plants were not studied.

One pale-flowered plant was observed among progeny descended from T321. This plant might have been the result of an outcross to a pale-flowered male parent. Seed of the pale-flowered plant were harvested but not studied.

Instances of seeming germinal instability of w4-dp could all be explained by natural outcrossing or by contamination (seed mixture). Some examples of seeming somatic instability of w4-dp might have epigenetic causes. Others might be more easily explained in terms of genetic instability. We conclude that w4-dp is very stable although we cannot rule out a low level of genetic instability.

References

Groose, R.W., S.M. Schulte and R.G. Palmer. 1990. Germinal reversion of an unstable mutation for anthocyanin pigmentation in soybean. Theor. Appl. Genet. 79:161-167.

Hartwig, E.E., and K. Hinson. 1962. Inheritance of flower color of soybeans. Crop Sci. 2:152-153.

Palmer, R.G., and R.W. Groose. 1993. A new allele at the w4 locus derived from the w4-m mutable allele in soybean. J. Hered. 84: In press.

Palmer, R.G., B.R. Hedges, R.S. Benavente and R.W. Groose. 1989. w4-mutable line in soybean. Develop. Genet. 10:542-551.

Weigelt, H.D., R.G. Palmer and R.W. Groose. 1990. Origin of the w4-m allele. Soybean Genet. Newsl. 17:81-84.

R. W. Groose
R. G. Palmer USDA-ARS

MAIZE RESEARCH INSTITUTE "ZEMUN POLJE"
Slobodana Bajica 1,11080 Zemun
Yugoslavia

Yugoslavia

Performance of AP6, AP12 and AP14 populations progeny

Introduction: Soybean germplasm populations with different percentage of germplasm from plant introduction to USA have been developed to provide germplasm for study in soybean breeding. In the current study, AP germplasm populations were used in an attempt to investigate the adaptability of progeny from broad genetic resources. Vello, Fehr and Bahrenfus (1984) evaluated genetic variability and agronomic performance of five populations with different percentages of PI germplasm: AP10(100%), AP11(75%), AP12(50%), AP13(25%), and AP14(0%). They concluded that the use of PI germplasm for short-term improvement of yield is not as productive as selection in population developed from domestic cultivars or experimental lines. Since genotype x environment interactions are important, it could be expected that germplasm selected for performance in USA will behave differently in the other countries. In this case, both PI and high yielding cultivars in AP germplasms are plant introductions to this country. Progeny from germplasm with more percentage of high yielding cultivars must not necessarily have higher yield when grown there.

Materials and methods: The S1 seed of AP6(S1), C1 and S2 seed of AP12 and AP14 germplasm populations was planted in 1986, at the location of Zemun Polje, Yugoslavia. The soybean population AP6 was developed from 40 high-yielding strains of Groups 0-IV maturity (Fehr and Ortiz, 1975). Three intermatings were applied and a diallel mating of 30 highest yielding lines to form AP6. Five more soybean germplasm populations named AP10 to AP14 were described by Fehr and Cianzio, 1981. These populations were developed from 40 plant introductions and 40 high yielding strains of Groups I and IV. The percentage of PI germplasm in these populations ranged from 0% to 100%.

The populations we planted, AP6 and AP14, had 100% high yielding strains germplasm, and AP12 had 50% germplasm from PI. The only criteria for selection in 1986 was maturity. Plants that matured until October 15 were harvested individually and the seed from each plant was sown in a separate row the next year. The pedigree

method of selection had been continued for the next three years until S4 lines from AP6 population and S5 lines from AP12 and AP14 populations were developed. Rows that showed high lodging, shattering, low number of pods and susceptibility to Diaporthe phaseolorum, Peronospora manshurica and Mottle virus were eliminated in every generation. Phytophthora megasperma was not observed, since it is of minor importance in these regions. The bulks from rows selected in 1988 were used for yield test of lines in 1989. So, visual selection was completed with yield data in this year. Selected superior lines with yield higher than adapted cultivar, Hodgson 78, were included into yield trial in 1990. Lines that overyielded Hodgson 78 in 1990 were included into yield trials in 1991 at two locations.

Results and discussion: The majority of every population we planted was of late maturity for this latitude (45°) and climatic characteristics of the region. The populations AP6 and AP12 had about 60% progeny planted in 1987. Very low numbers of progeny, only 18 rows, were selected in the population AP14.

The best performance in 1987 showed AP6 with 49 selected lines, which was 41.2% of planted material. Only eight S4 lines were selected from AP14, although it was 44.4% selection intensity.

The population AP6 proved to have the widest adaptability compared with other two AP populations. Seven superior lines were selected from this population on the basis of their performance in 1989. The population AP12 showed lower adaptability, having two superior lines. It turned out that AP14 germplasm was not adaptive for this region, because no line was selected from this population. The main disadvantages of observed soybean populations were late maturity, lodging and susceptibility to Diaporthe phaseolorum var caulivora. Selected lines did not pass yield trials because none of them had higher yield in all three years compared with adapted cultivar, Hodgon 78.

Results presented here lead to the conclusion that intermated populations derived from broad genetic resources were not adaptive enough in a region that differs from the place where these populations were developed. Selected progeny in a short term pedigree method could not compete with cultivar already adapted here.

Schoener and Fehr (1979) evaluated the relationship between percentage of germplasm from PI in five soybean populations, AP1 to AP5 and their performance for

seed yield, height and lodging. The percentage of PI germplasm was from 100% in AP1 to 0% in AP5. Their results indicated that, for immediate use of a population for yield improvement there is little advantage for using any plant introduction parent. AP5 had the highest mean yield and considerable amount of genetic variability. The average superiority of parental lines from AP14 in the research that Vello, Fehr and Bahrenfus (1984) conducted was related to the higher frequency of high-yielding lines in that population as compared with other AP populations. Lodging susceptibility increased as the percentage of PI germplasm in the populations increased. If we consider all three germplasms, AP6, AP12 and AP14, in our investigation as plant introduction germplasms, our results confirm the conclusions of Shoener and Fehr (1979) and Vello, Fehr and Bahrenfus (1984).

Martin and Aslam (1986) reported that F3 bulks derived from PI parents yielded less than those of adapted, and were more lodging susceptible. Khalaf, Brossman and Wilcox (1984) evaluated the lines derived from crosses among three cultivars and three plant introductions. Three-parent crosses, with the highest proportion of adapted germplasm had higher yield than those with lower proportion of adapted germplasm.

A recurrent selection program that included nine plant introductions crossed to a highly productive experimental line gave favorable yield response as reported by Kenworthy and Brim (1979). This breeding scheme supports the idea that soybean populations of greater diversity and productivity can be developed using plant introductions.

Table 1. Successive generations of AP germplasm populations.

	1986		1987		1988		1989	
Populat.	Gener.	No. planted	Gener.	No. of rows	Gener.	No. of rows	Gener.	No. of lines
AP6	S1	200	S2	119	S3	49	S4	18
AP12	S2	200	S3	122	S4	25	S5	7
AP14	S2	200	S3	18	S4	8	S5	2

Table 2. Yield testing of progeny from AP germplasm populations.

Population	Number of tested lines			No. of lines overyielded standard in all 3 years
	1989	1990	1991	
AP6	18	7	3	0
AP12	7	2	1	0
AP14	2	0	0	0

Table 3. Average yield of lines from AP germplasm populations.

Population	Yield calculated to kg/ha			
	1989	1990	1991	
			Location 1	Location 2
AP6	3000	2116	2438	3860
AP12	2383	2175	2625	-
AP14	2335	-	-	-
Hodgson 78	2823	2050	3065	4460

References:

Fehr, W.R. and L.B. Ortiz. 1975. Registration of a soybean germplasm population. Crop Sci. 15:739.

Fehr, W.R. and S.R. de Cianzio. 1981. Registration of soybean germplasm populations AP10 to AP14. Crop Sci. 21:477-478.

Kenworthy, W.J. and C.A. Brim. 1979. Recurrent selection in soybeans. 1. Seed yield. Crop Sci. 19:315-318.

Khalaf, A.G.M., G.D. Brossman and J.R. Wilcox. 1984. Use of diverse populations in soybean breeding. Crop Sci. 24:358-360.

Schoener, C.S. and W.R. Fehr. 1979. Utilization of plant introduction in soybean breeding populations. Crop Sci. 19:185-188.

St. Martin, S.K. and M. Aslam. 1986. Performance of progeny of adapted and plant introduction soybean lines. Crop Sci. 26: 753-756.

Vello, N.A., W.R. Fehr and J.B. Bahrenfus. 1984. Genetic variability and agronomic performance of soybean populations developed from plant introductions. Crop Sci. 24:511-514.

L.M. Prijic

MAIZE RESEARCH INSTITUTE ZEMUN POLJE
11080 Zemun, Slobodana Bajica 1
Yugoslavia

24

Effects of grafting date on flowering of soybean scions

It is often a problem how to multiply seeds of late cultivars when maintaining germplasm collections. Seeds of such cultivars do not mature before autumn frosts. Making cross-pollinations among early and late parents is sometimes a problem in breeding programs because flowering time differs. One possible way to get early flowering of late soybean in a season could be early planting in a greenhouse, combined with grafting late cultivar on an early one. It was supposed that grafted late cultivar would enhance its development upon the influence of stock plant (early cultivar).

Materials and methods: The soybean cultivar, Fiskeby, maturity group 000, was used as a stock plant. Three late maturity indeterminate soybean cultivars of diverse origin were used as grafting material: cultivars Kaleya and Tunia were from Africa (Zambia) and third one , Grunkong, was from Korea.

The first planting date was on March 8 and the second was 15 days later, on March 23 in the greenhouse. Grafting was done on March 28 and April 7, respectively. Six seeds were planted per pot. Three uniform plants in every pot were left as stock plants for grafting. There were 12 stock plants (four pots) for each variant. Plants that had to be used for making grafting scions were planted in separate pots at the same time. Grafting was done in vegetative stage, V2 (Fehr, 1979), when the stock plants had one developed trifoliate leaf. A graft scion was formed by cutting the plant top under the first node with a developed leaf. The lower end of a scion was formed as a wedge. Leaflets were trimmed to half size to decrease transpiration. The top of a stock plant was removed by cutting. Grafting was done in two modes, according to the place where the stock plant was cut and made vertical fissure where the scion had to be incorporated. First, the scion was placed into the fissure at first unifoliate leaf node level. The grafted place was firmly tied and the plant covered with a transparent PVC bag. These bags were left on the plants for ten days.

Development of grafted soybean was monitored by recording when the grafts

reached the reproductive stages defined by Fehr and Cavines (1979) until stage R6 (full seed).

Environmental conditions during experiment

Daylength and temperature play important roles in soybean development. These factors can be crucial in determining the beginning of flowering and duration of subsequent reproductive stages. Average daylength and daily temperatures in a greenhouse during observed period are presented in Table 1.

In the observed period March-July at latitude 44, 5 average decade daylength is in a range room from 11, 3 (first decade of March) to 15, 6 hours (last decade of June). Reproductive stages R1 to R6 of soybean in this experiment were in period May-July, so reproductive development occurred in a long day term. There was a cold period in the first decade of May. This prolonged vegetative development of soybean.

Results:

Percentage of successful graftings

Percentage of successful graftings was very high (Table 2). Soybean showed 94% successful graftings when grafted at cotyledon node level, and 97% when grafted at first unifoliate leaf level. Sufficient callus swelling was noticed when grafting was done at cotyledon node level.

Development of grafted soybean

Main interest in this investigation was to enhance flowering (stage R2) by means of grafting. Reproductive development of grafted soybean is presented in Figures 1 and 2. Grafting mode did not effect reproductive development of soybean, so figures are referring to average data for both grafting modes.

Cultivars Tunia and Kaleya did not manage to flower without grafting in both planting dates. Daylength was probably inadequate for these cultivars. Flowers of grafted Tunia were abortive in R2 stage in both planting dates.

Grafted Kaleya reached R2 stage 79 days after first planting date and 76 days after the second planting date. Subsequent stages appeared 2-9 days earlier in the second planting date compared with the first planting date when number of days from planting was counted.

Cultivar Grunkong originates from latitudes more similar to the latitude of the site of the experiment, so daylength was more favorable for reproductive development of this cultivar. Grunkong flowered (R2 stage) after 79 days in control variant of first planting date and after 70 days in control variant of second planting date. Grafted Grunkong reached R2 stage after 68 and 64 days, respectively. That is 11 and 6 days earlier when compared with nongrafted plants. The difference of 1-2 weeks in appearance of reproductive stages between grafted and nongrafted Grunkong was present through the observed period.

Conclusion: Late cultivars were grafted on very early ones with high percentage of successful graftings. Due to grafting, flowering appeared 1-2 weeks earlier.

Late maturity cultivars could be forced to flower earlier in a season by combining early planting in a greenhouse and grafting on very early cultivars. This would be useful in germplasm collections for seed multiplication of late cultivars and in breeding procedure to match flowering of early and late parents.

References

Fehr, W.R., Caviness, C.E. (1979): Stages of Soybean Development. Spec.Report 80. Iowa State University.

Ljubisa Prijic

Table 1. Average daylength and daily temperatures (°C in the greenhouse) during the observed period

Month	Decade	Day length h	Average daily temp. °C
March	I	11, 3	14, 5
	II	11, 9	19, 0
	III	12, 4	23, 3
April	I	13, 0	23, 4
	II	13, 4	20, 6
	III	13, 9	22, 0
May	I	14, 4	14, 9
	II	14, 8	22, 3
	III	15, 2	22,5
June	I	15, 5	22, 9
	II	15, 6	20, 7
	III	15, 6	25, 8
July	I	15, 5	30, 0
	II	15, 2	24, 4
	III	14, 9	25, 5

Table 2. Percentage of successful graftings

Grafted cultivar	Planting date	Successful graftings %	
		At cotyledon node	At first unifoliate leaf
Kaleya	8 March	100	100
	23 March	100	100
Tunia	8 March	83	100
	23 March	92	83
Grunkong	8 March	92	100
	23 March	100	97
Average		94	97

Fig. 1:Reproductive development of grafted soyban in first planting date

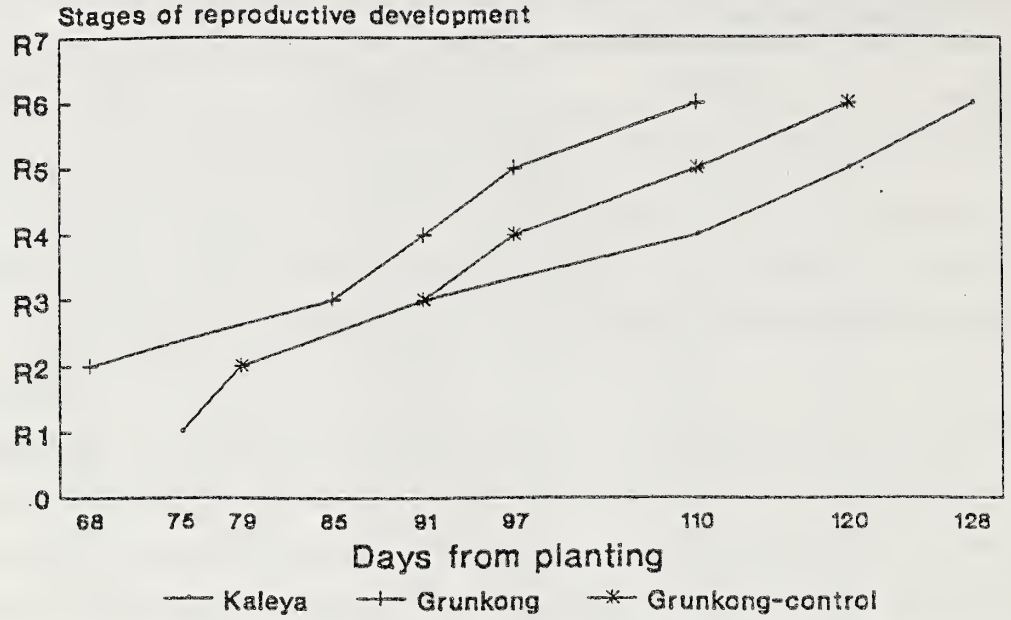
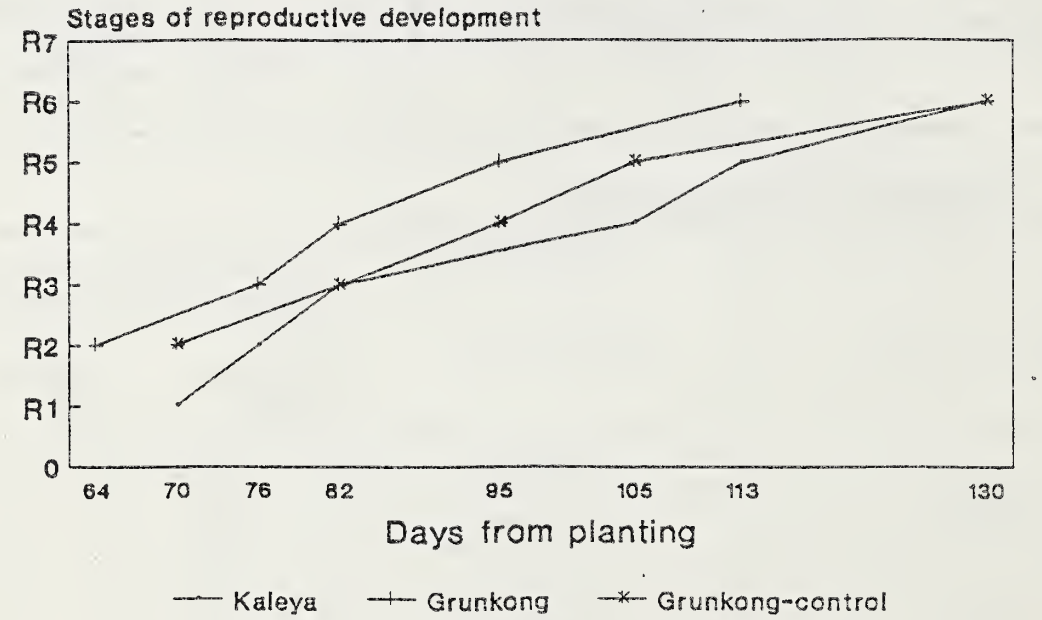


Fig.2:Reproductive development of grafted soybean in second planting date



Index of Authors